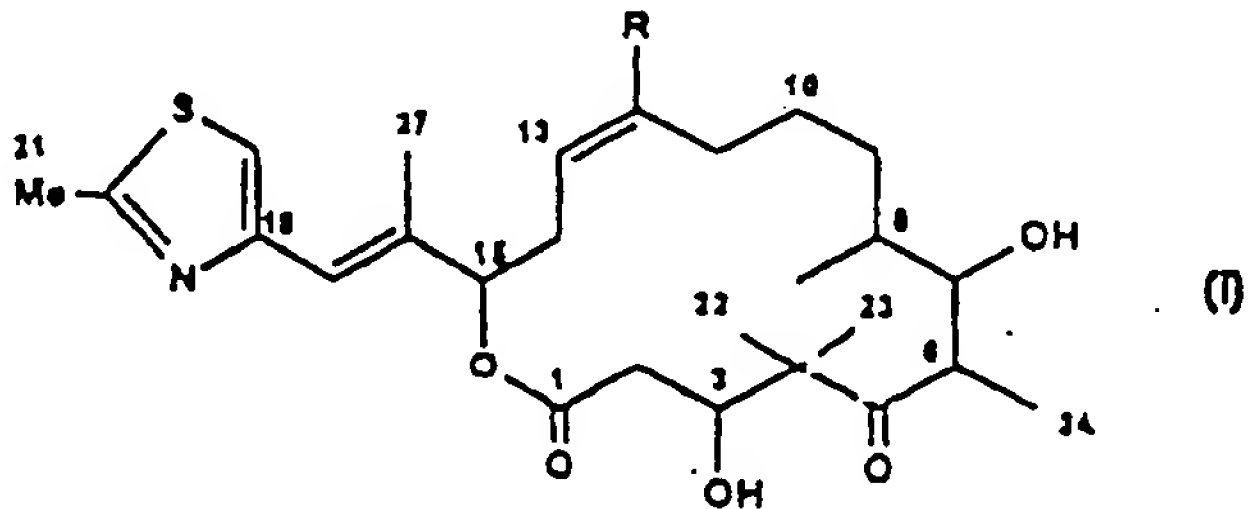
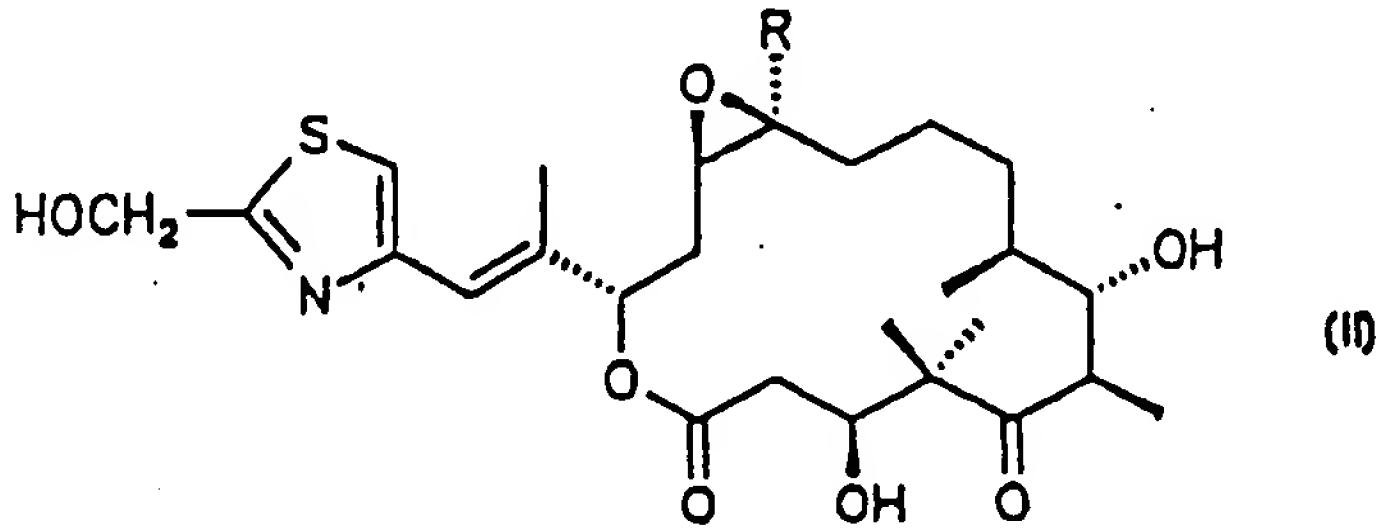


PCT
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<p>(21) Internationales Aktenzeichen: PCT/EP97/06442</p> <p>(22) Internationales Anmeldedatum: 18. November 1997 (18.11.97)</p> <p>(30) Prioritätsdaten: <div style="display: flex; justify-content: space-between;"> <div>196 47 580.5</div> <div>18. November 1996 (18.11.96)</div> <div>DE</div> </div> <div style="display: flex; justify-content: space-between;"> <div>197 07 506.1</div> <div>25. Februar 1997 (25.02.97)</div> <div>DE</div> </div> </p> <p>(71) Anmelder (für alle Bestimmungsstaaten ausser US): GESELLSCHAFT FÜR BIOTECHNOLOGISCHE FORSCHUNG MBH (GBF) [DE/DE]; Mascheroder Weg 1, D-38124 Braunschweig (DE).</p> <p>(72) Erfinder; und (75) Erfinder/Anmelder (nur für US): REICHENBACH, Hans [DE/DE]; Mascheroder Weg 1, D-38124 Braunschweig (DE). HÖFLE, Gerhard [DE/DE]; Mascheroder Weg 1, D-38124 Braunschweig (DE). GERTH, Klaus [DE/DE]; Mascheroder Weg 1, D-38124 Braunschweig (DE). STEIN-METZ, Heinrich [DE/DE]; Mascheroder Weg 1, D-38124 Braunschweig (DE).</p> <p>(74) Anwälte: BOETERS, Hans, D. urw.; Boeters & Bauer, Bereiteranger 15, D-81541 München (DE).</p>		
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<p>(54) Title: EPOTHILONE C, D, E AND F, PRODUCTION PROCESS, AND THEIR USE AS CYTOSTATIC AS WELL AS PHYTOSANITARY AGENTS</p> <p>(54) Bezeichnung: EPOTHILONE C, D, E UND F, DEREN HERSTELLUNG UND DEREN VERWENDUNG ALS CYTOSTATISCHE MITTEL BZW. ALS PFLANZENSCHUTZMITTEL</p>		
<p>(57) Abstract</p> <p>The present invention concerns the epothilone, especially epothilone C (R = hydrogen) and epothilone D (R = methyl) of formula (I), as well as epothilone E (R = hydrogen) and epothilone F (R = methyl) of formula (II), the production process and their application for producing therapeutic agents, including cytostatic agents, as well as phytosanitary agents.</p> <p>(57) Zusammenfassung</p> <p>Die vorliegende Erfindung betrifft Epothilone, insbesondere Epothilon C (R = Wasserstoff) und Epothilon D (R = Methyl) der Formel (I) sowie Epothilon E (R = Wasserstoff) und Epothilon F (R = Methyl) der Formel (II), deren Herstellung, sowie deren Verwendung zur Herstellung von therapeutischen, insbesondere cytostatischen Mitteln sowie Mitteln für den Pflanzenschutz.</p>		
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BOETERS & BAUER
Patent Attorneys Letterhead

November 17, 1997/he

Our reference: 8824-GBF

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based on DE (1) 96 47 580.5 and DE (1) 97 07 506.1

Gesellschaft für Biotechnologische Forschung mbH (GBF)

Epothilone C, D, E and F, Preparation and Agents

The present invention is concerned with epothilone C, D, E and F, with their preparation as well as with their application for the preparation of therapeutic agents and agents for plant protection.

Epothilone C and D

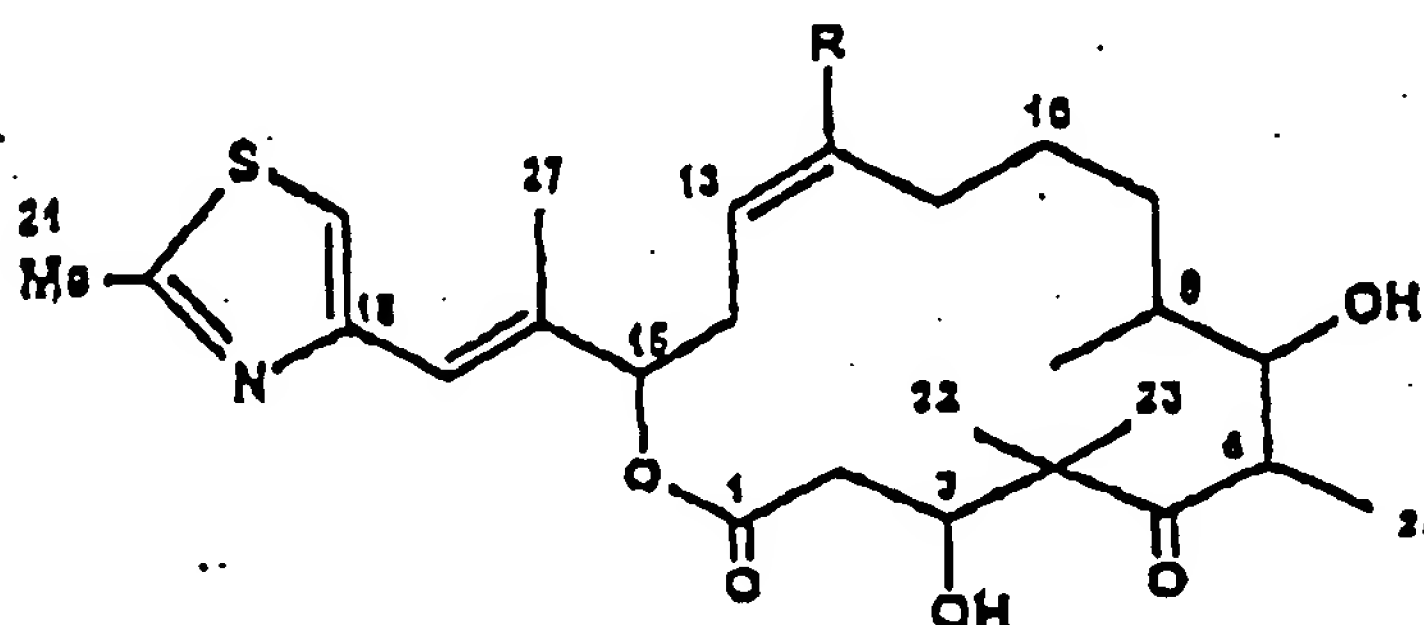
According to an embodiment, the invention is concerned with Epothilone C and D, which can be obtained by

- (a) cultivating *Sorangium cellulosum* DSM 6773 in the presence of an adsorber resin in the known manner,
- (b) the adsorber resin is separated from the culture and is washed with a water/methanol mixture,
- (c) the washed adsorber resin is eluted with methanol and the eluate is evaporated to obtain a crude extract,
- (d) the obtained concentrate is extracted with ethyl acetate, the extract is evaporated and partitioned between methanol and hexane,
- (e) the methanolic phase is evaporated to a raffinate and the concentrate is fractionated on a Sephadex column,
- (f) a fraction is obtained with metabolic products of the microorganism used,
- (g) the obtained fraction is chromatographed on a C18-reverse-phase with a methanol/water mixture and the following are obtained in a time sequence,
 - after a first fraction with epothilone A and
 - a second fraction with epothilone B,
 - a third fraction with first additional epothilone and
 - a fourth fraction with a second additional epothilone are obtained and
- (h1) the epothilone of the additional fraction and/or

(h2) the epothilone of the second additional fraction is isolated.

Furthermore the invention is concerned with epothilone [C] having the molecular formula $C_{26}H_{39}NO_5S$, characterized by the 1H and ^{13}C -NMR spectrum according to Table 1.

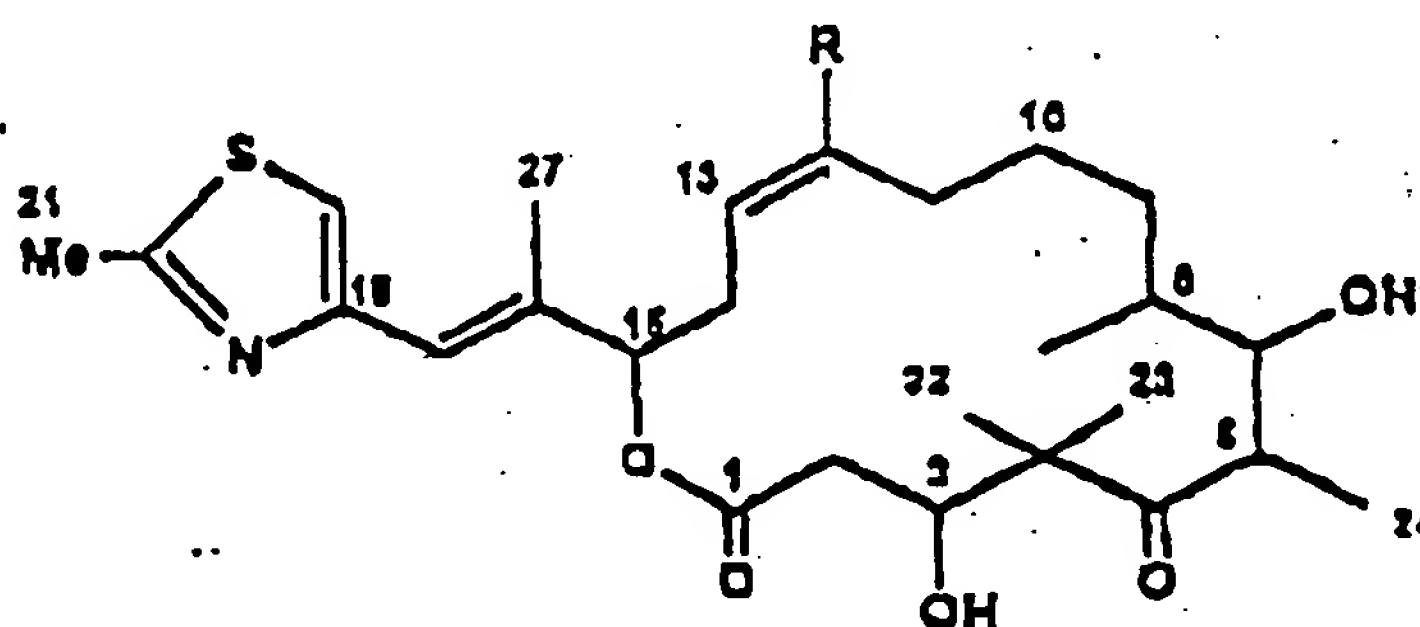
Furthermore, the invention is concerned with epothilone C having the formula:



epothilone C R = H

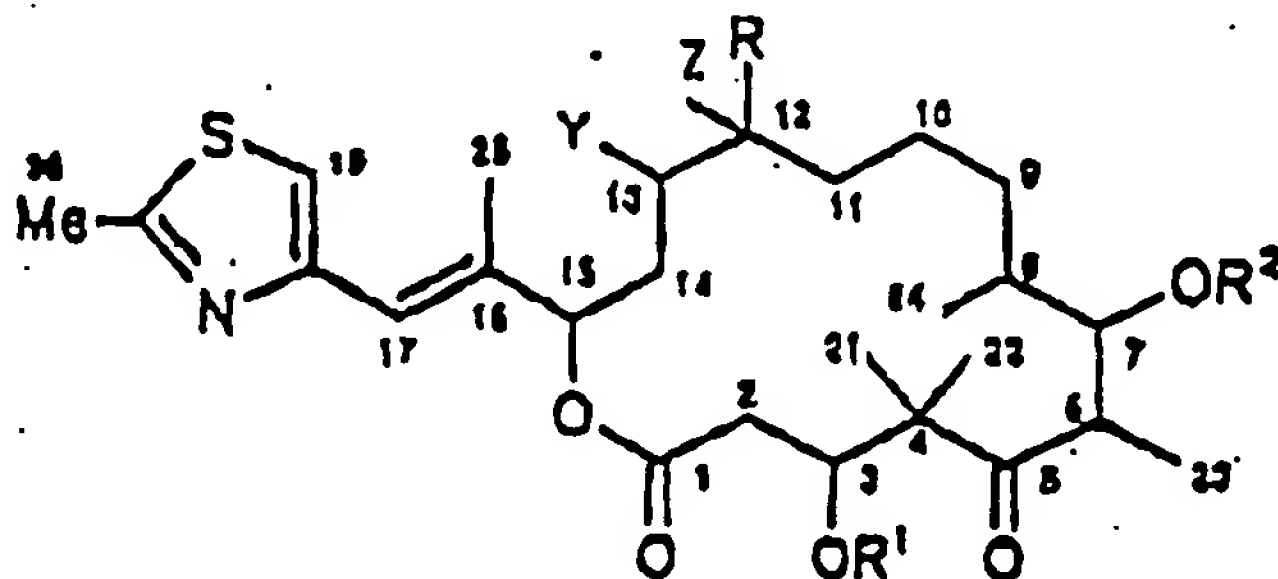
Furthermore, the invention is concerned with epothilone [D] having the molecular formula $C_{27}H_{41}NO_5S$, characterized by the 1H and ^{13}C -NMR spectrum according to Table 1.

Furthermore, the invention is concerned with epothilone D having the formula:



epothilone D R = CH₃

Epothilone C and D can be used for the preparation of compounds having the following Formula 1 and, with regard to their derivatization, reference can be made to the derivatization methods described in WO-A-97/19 086.



In the above formula 1, the symbols have the following meaning:

R = H, C₁₋₄-alkyl;

R¹, R², R³, R⁴, R⁵ = H, C₁₋₆-alkyl,

C₁₋₆ acyl benzoyl,

C₁₋₄ trialkylsilyl,

benzyl,

phenyl,

C₁₋₆ alkoxy-,

C₆-alkyl, hydroxy and halogen-substituted benzyl or phenyl;

but two of the groups R¹ to R⁵ can be combined to form the group -(CH₂)_n- with n = 1 to 6, and the alkyl and acyl groups contained in these groups are straight-chain or branched groups;

Y and Z are either the same or different and each stands for hydrogen, halogen, such as F, Cl, Br or I, pseudohalogen, such as -NCO, -NCS or -N₃, OH, O-(C₁₋₆)-acyl, O-(C₁₋₆)-alkyl, O-benzoyl, Y and Z can also be the O-atom of an epoxide, but epothilone A and B are not claimed, or one of the C-C bonds can be a C=C double bond.

Thus, the 12,13-double bond can be selectively

- hydrogenated, for example, catalytically or with diimine, obtaining a compound having formula 1 with Y = Z = H; or

- epoxidized, for example, with dimethyldioxirane or a peracid, obtaining a compound having Formula 1 with Y with Z = -O-; or

- inverted to the aziridines, dipseudohalides or diazides, obtaining a compound having formula 1 with Y and Z = halogen, pseudohalogen or N₃.

Epothilone E and F

According to another embodiment, the invention is concerned with a biotransformant of epothilone A, which can be obtained by:

- (a) cultivating *Sorangium cellulosum* DSM 6773 in the presence of an adsorber resin in the known manner, separating it from the adsorber resin and optionally adding a methanolic solution of epothilone A to the total amount or to a part of the separated culture,
- (b) the culture to which the epothilone A was added is incubated and then adsorber resin is added,
- (c) the adsorber resin is separated from the culture, eluted with methanol and the eluate is evaporated to a crude extract,
- (d) the crude extract is partitioned between ethyl acetate and water, the ethyl acetate phase is separated and evaporated to an oil,
- (e) the oil is chromatographed on a reverse phase under the following conditions:
column material: Nucleosil 100 C-18 7 μ m
column dimensions: 250 x 16 mm
solvent: methanol/water = 60:40
flow rate: 10 mL/min

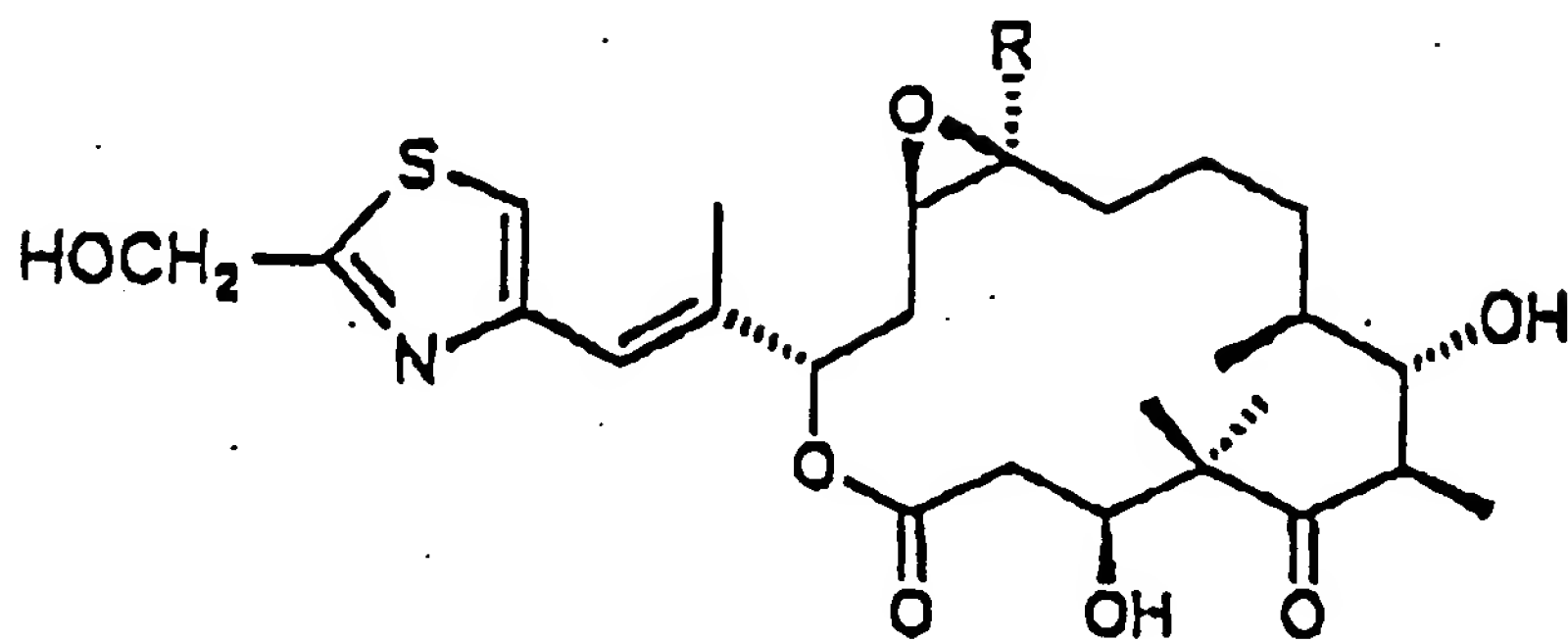
and fractions with a content of biotransformant, which can be detected by UV adsorption at 254 nm, with an R_f value of 20 minutes, is separated and the biotransformant is isolated.

Furthermore, the invention is concerned with such a biotransformant of epothilone A, which can be obtained by separating a culture in step (a), which is three or four days old or more.

Furthermore, the invention is concerned with such a biotransformant of epothilone A, which can be obtained by incubating step (b) one or two or more days.

Furthermore, the invention is concerned with a compound having the molecular formula $C_{26}H_{39}NO_7S$, characterized by the following 1H -NMR spectrum (300 MHz, $CDCl_3$):

Furthermore, the invention is concerned with a compound (epothilone E) having the following formula:



epothilone E R = H

According to a further embodiment, the invention is concerned with a biotransformant of epothilone B, which can be obtained by

- (a) cultivating *Sorangium cellulosum* DSM 6773 in the presence of an adsorber resin in the known manner, separating it from the absorber resin and optionally the total amount or a part of the separated culture is treated with a methanolic solution of epothilone B,
- (b) the culture to which the epothilone B was added is incubated and then adsorber resin is added,
- (c) the adsorber resin is separated from the culture, eluted with methanol and the eluate is evaporated to give a crude extract,
- (d) the crude extract is partitioned between ethyl acetate and water, the ethyl acetate phase is separated and evaporated to an oil,
- (e) the oil is chromatographed on a reverse-phase under the following conditions:
column material: Nucleosil 100 C-18 7 μ m
column dimensions: 250 x 16 mm
solvent: methanol/water = 60:40
flow rate: 10 mL/min

and fractions of biotransformant content that can be detected by UV absorption at 254 nm, with an R_f value of 24.5 min are separated and the biotransformant isolated.

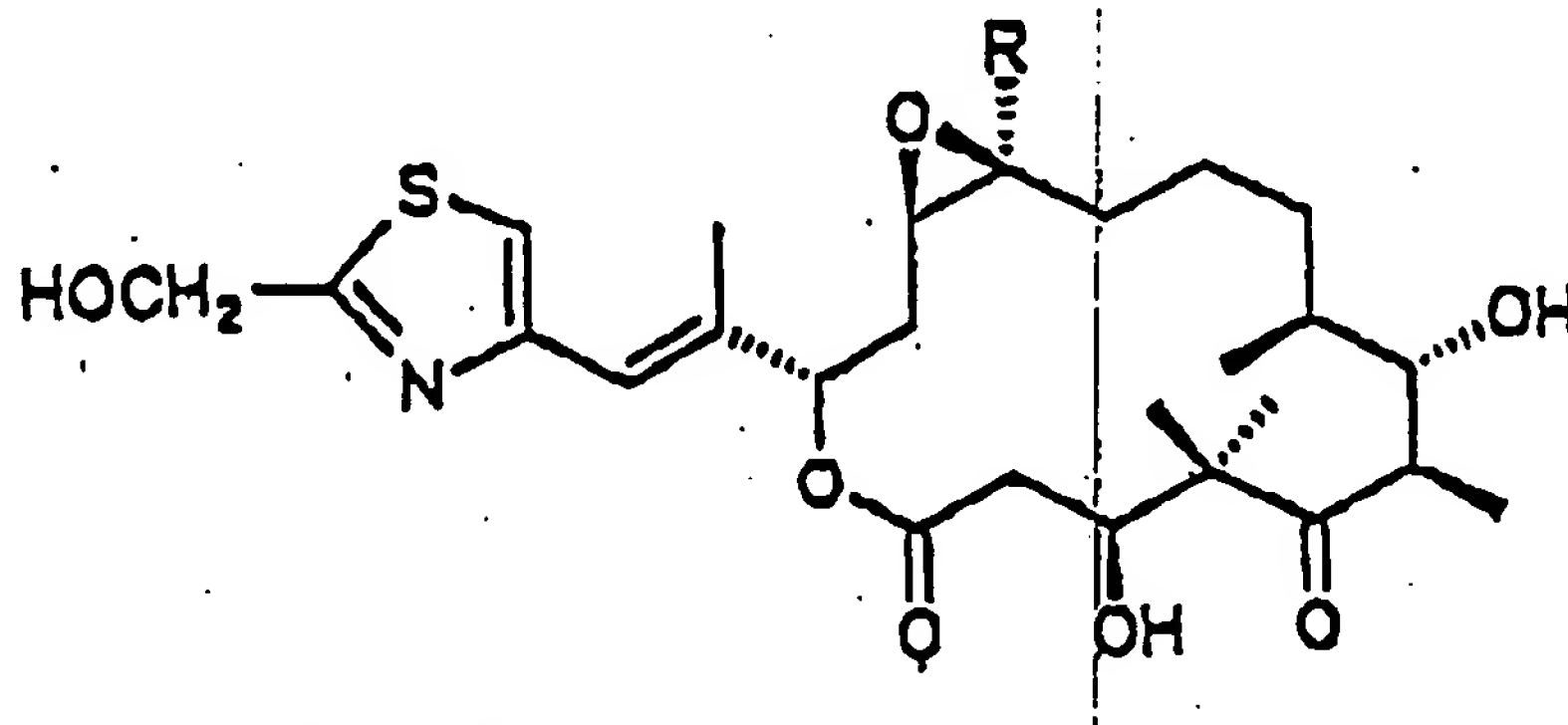
Furthermore, the invention is concerned with such a biotransformant of epothilone B, which can be obtained by separating at step (a) a culture which is three or four or more days old.

Furthermore, the invention is concerned with such a biotransformant of epothilone B, which can be obtained by incubation at step (b) for one or two or more days.

Furthermore, the invention is concerned with a compound having the molecular formula $C_{27}H_{41}NO_7S$, characterized by the following 1H -NMR spectrum (300 MHz, $CDCl_3$):

δ = 2.37 (2- H_a), 2.52 (2- H_b), 4.20 (3-H), 3.27 (6-H), 3.74 (7-H), 1.30 - 1.70 (8-H, 9- H_2 , 10- H_2 , 11- H_2), 2.78 (13-H), 1.91 (14-H), 2.06 (14- H_b), 5.42 (15-H), 6.58 (17-H), 7.10 (19-H), 4.89 (21- H_2), 1.05 (22- H_3), 1.26 (23- H_3), 1.14 (24- H_3), 0.98 (25- H_3), 1.35 (26- H_3), 2.06 (27- H_3).

Furthermore, the invention is concerned with a compound (epothilone F) having the formula:



epothilone F R = CH_3

Preparation and means

The compounds according to the invention or epothilones can be obtained with the measures listed above.

Furthermore, the invention is concerned with means for plant protection in agriculture, forestry and/or gardening, consisting of one or more of epothilones C, D, E and F listed above or consisting of ~~any~~ ^{some} of the epothilones listed above in addition to one or several usual carrier(s) ~~or~~ ^{and} diluent(s).

Finally, the invention is concerned with therapeutic agents consisting of one or more of the compounds listed above or one or more of the compounds listed above together with one or more of common carrier(s) and/or diluent(s). These means can exhibit especially cytotoxic activities and/or cause immune suppression and/or can be used for the combatting of malignant tumors, where they are used especially preferably as cytostatic agents.

The invention is explained in more detail and described below by the description of some selected practical examples.

Examples

Example 1

Epothilone C and D

A. Product strain and culture conditions corresponding to epothilone basic patent DE-B-41 38 042

B. Production with DSM 6773

75 L of culture is raised as described in the basic patent and used for inoculating a production fermenter with 700 L production medium consisting of 0.8% starch, 0.2% glucose, 0.2% soy meal, 0.2% yeast extract, 0.1% $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.1% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 8 mg/L of Fe-EDTA, pH = 7.4 and optionally 15 L of adsorber resin Amberlite XAD-16. The fermentation takes 7-10 days at 30°C, with aeration at 0.1 NL/m³. The pO₂ is kept at 30% by adjusting the rate of rotation.

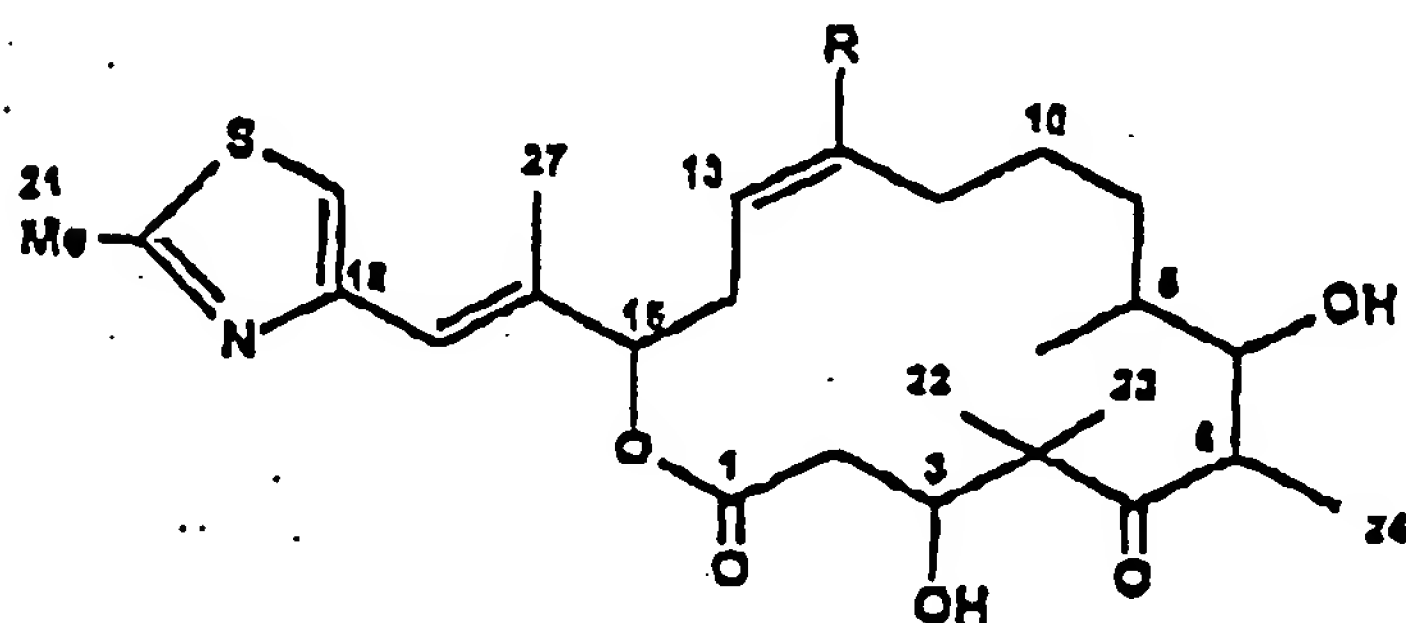
C. Isolation

The adsorber resin is separated from the culture with a 0.7 m², 100 mesh process filter and the polar accompanying substances are removed by washing with 3 bed volumes of water/methanol 2:1. A crude extract is obtained by elution with 4 bed volumes of methanol. This is evaporated in vacuum until the appearance of the aqueous phase.

This is then extracted three times with the same volume of ethyl acetate. Evaporation of the organic phase gives 240 g of crude extract, which is partitioned between methanol and heptane in order to separate lipophilic accompanying substances. Then 180 g of raffinate is obtained from the methanol phase by evaporation in vacuum. This is fractionated into three

portions on Sephadex LH-20 (column 20 x 100 cm, 20 mL/min of methanol). The epothilones are contained in the fraction eluted at a retention time of 240-300 min in a total amount of 72 g. To separate the epothilones, the product is chromatographed in three portions on Lichrosorb RP-18 (15 μ m, column 10 x 40 cm, solvent 180 mL/min methanol/water 65:35). After the epothilone A and B, epothilone C and epothilone D are eluted at R_f = 90-95 min and 100-110 min, respectively. After evaporation in vacuum, each is obtained in a yield of 0.3 g as colorless oils.

D. Physical properties



epothilone C $R = H$
 epothilone D $R = CH_3$

Epothilone C

$C_{26}H_{39}NO_5S$ [477]

ESI-MS: (positive ions): 478.5 for $[M+H]^+$

1H and ^{13}C see NMR Table.

TLC: $R_f = 0.82$

TLC: aluminum foil 60 F 254 Merck, solvent: dichloromethane/methanol = 9:1

Detection: UV absorption at 254 nm. Spraying with vanillin-sulfuric acid reagent, blue-gray coloration upon heating to 120°C.

HPLC: $R_f = 11.5$ min

Column: Nucleosil 100 C-18 7 μ m, 125 x 4 mm

Solvent: methanol/water = 65:35

Flow rate: 1 mL/min
Detection: diode array

Epothilone D

$C_{27}H_{41}NO_5S$ [491]

ESI-MS: (positive ions): 492.5 for $[M+H]^+$

1H and ^{13}C , see NMR Table

TLC: $R_f = 0.82$

TLC: aluminum foil 60 F 254 Merck, solvent: dichloromethane/methanol = 9:1

Detection: UV absorption at 254 nm. Spraying with vanillin-sulfuric acid reagent, blue-gray coloration upon heating to 120°C.

HPLC: $R_t = 15.3$ min

Column: Nucleosil 100 C-18 7 μm , 125 x 4 mm

Solvent: methanol/water = 65:35

Flow rate: 1 mL/min

Detection: diode array

Table 1: ^1H and ^{13}C -NMR data of epothilone C and epothilone D in $[\text{D}_6\text{DMSO}]$ at 300 MHz

Epothilone C				Epothilone D			
H-Atom	δ (ppm)	C-Atom	δ (ppm)	δ (ppm)	C-Atom	δ (ppm)	
		1	170.3		1	170.1	
2-Ha	2.38	2	38.4	2.35	2	39.0	
2-Hb	2.50	3	71.2	2.38	3	70.8	
3-H	3.97	4	53.1	4.10	4	53.2	
3-OH	5.12	5	217.1	5.08	5	217.4	
6-H	3.07	6	45.4	3.11	6	44.4	
7-H	3.49	7	75.9	3.48	7	75.5	
7-OH	4.46	8	35.4	4.46	8	36.3	
8-H	1.34	9	27.6	1.29	9	29.9	
9-Ha	1.15	10	30.0	1.14	10	25.9	
9-Hb	1.40	11	27.6	1.38	11	31.8*	
10-Ha	1.15*	12	124.6	1.14*	12	138.3	
10-Hb	1.35*	13	133.1	1.35*	13	120.3	
11-Ha	1.90	14	31.1	1.75	14	31.6*	
11-Hb	2.18	15	76.3	2.10	15	76.6	
12-H	5.38**	16	137.3		16	137.2	
13-H	5.44**	17	119.1	5.08	17	119.2	
14-Ha	2.35	18	152.1	2.30	18	152.1	
14-Hb	2.70	19	117.7	2.65	19	117.7	
15-H	5.27	20	164.2	5.29	20	164.3	
17-H	6.50	21	18.8	6.51	21	18.9	
19-H	7.35	22	20.8	7.35	22	19.7	
21-H ₂	2.65	23	22.6	2.65	23	22.5	
22-H ₂	0.94	24	16.7	0.90	24	16.4	
23-H ₂	1.21	25	18.4	1.19	25	18.4	
24-H ₂	1.06	27	14.2	1.07	26	22.9	
25-H ₂	0.90			0.91	27	14.1	
26-H ₂				1.63			
27-H ₂	2.10			2.11			

*, ** Assignment interchangeable

Example 2:

Epothilone A and 12,13-bisepi-epothilone A from epothilone C

Epothilone A, 50 mg, is dissolved in 1.5 mL acetone and 1.5 mL of a 0.07 molar solution of dimethyldioxiran in acetone is added. After standing for 6 hours at room temperature, the

mixture is evaporated in vacuum and separated by preparative HPLC on silica gel (solvent: methyl-tert.-butyl ether/petroleum ether/methanol 33:66:1).

Yield:

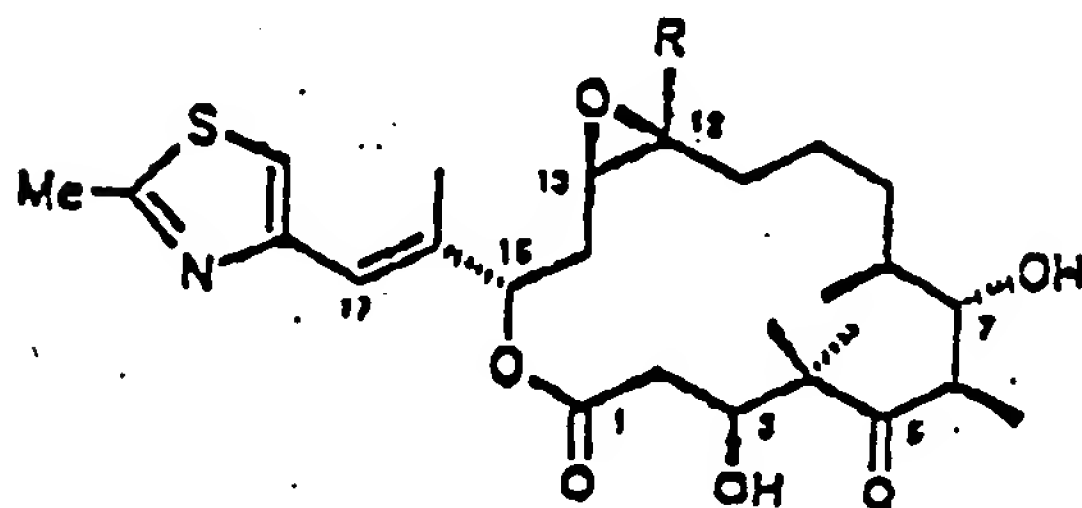
25 mg of epothilone A, $R_f = 3.5$ min (analytical HPLC, $7\ \mu\text{m}$, column 4×250 mm, solvent see above, flow rate 1.5 mL/min)

and

20 mg of 12,13-bisepi-epothilone A, $R_f = 3.7$ min, ESI-MS (positive ions)

$m/z = 494 [M+H]^+$

$^1\text{H-NMR}$ in $[\text{D}_4]$ methanol, selected signals: $\delta = 4.32$ (3-H), 3.79 (7-H), 3.06 (12-H), 3.16 (13-H), 5.54 (15-H), 6.69 (17-H), 1.20 (22-H), 1.45 (23-H).



12,13-bisepi-epothilone A

$R = \text{H}$

Example 3:

Epothilone E and F, new biotransformation products of epothilones A and B

Production strain:

The production strain, *Sorangium cellulosum* So ce90, was isolated from a soil sample collected in July 1985 at the GBF at the banks of the Zambesi and was deposited on 10/28/91 in the Deutsche Sammlung für Mikroorganismen [German Collection for Microorganisms] under No. DSM 6773.

The characterization of the producing organism as well as the culturing conditions are described in:

Höfle, G.; N. Bedorf, K. Gerth & H. Reichenbach: Epothilones, their methods of preparation as well as agents containing them. DE 41 38 042 A1, laid open on May 27, 1993.

Formation of epothilones E and E during fermentation: [probably should be epothilones A and E - Translator]

A typical fermentation runs as follows: a 100 L bioreactor is filled with 60 L medium (0.8% starch; 0.2% glucose; 0.2% soy meal; 0.2% yeast extract; 0.1% $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$; 0.1% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$; 8 mg/L of Fe-EDTA; pH 7.4). In addition, 2% adsorber resin (XAD-16, Rohm & Haas) was added. The medium is sterilized by autoclaving (2 hours, 120°C). Inoculation is done with 10 L of a preculture raised in a shaking flask in the same medium (in addition: 50 mM HEPES buffer pH 7.4) (160 rpm, 30°C). The fermentation is carried out at 32°C at a stirrer velocity of 500 rpm and aeration with 0.2 NL per m^3 and hour. The pH value is kept at 7.4 by the addition of KOH. The fermentation takes 7 to 10 days. The formed epothilones are bound to the adsorber resin continuously during the fermentation. After separation of the culture broth (for example, by sieving in a process filter) the resin is washed with 3 bed volumes of water and eluted with 4 bed volumes of methanol. The eluate is evaporated to dryness and is taken up in 700 mL of methanol.

HPLC analysis of the XAD eluate:

With respect to the initial volume of the reactor (70 L), the eluate is concentrated 100:1. The analysis is carried out with an HPLC unit 1090 made by Hewlett Packard. A Microbore column (125/2 Nucleosil 120-5 C_{18}) made by Machery-Nagel (Düren) is used for separating the components. The elution is done with a water/acetonitrile gradient from initially 75:25 to 50:50 after 5.5 minutes. This ratio is then maintained till the 7th minute and then increased to 100% acetonitrile up to the 10th minute.

The measurement is carried out at a wavelength of 250 nm and with a band width of 4 nm. The diode array spectra are measured in the wavelength region from 200 to 400 nm. In the XAD eluate, two new substances are noticed with R_f of 5.29 and R_f of 5.91; the adsorption spectra of these are identical to those of epothilones A and B, respectively (Figure 1; e corresponds to A, F corresponds to B). These substances are formed only in traces under the given fermentation conditions.

Biotransformation of epothilone A and B to epothilone E and F:

For the directed biotransformation, a 4-day old culture of So ce90, 500 mL, is used, kept with adsorber resin. Of this, 250 mL is introduced into a sterile 1 L Erlenmeyer flask leaving the XAD behind. After that, a methanolic solution of a mixture of a total of 36 mg of epothilone A and 14 mg of epothilone B is added and the flask is incubated for two days at 30°C and 200 rpm on a shaking chest [literal]. The formation of epothilone E and F is analyzed directly on 10 μ L of the centrifuged culture supernatant (Figure 2). The conversion occurs only in the presence of the cells and is dependent on the cell density used and on the time. The kinetics of conversion for epothilone A is shown in Figure 3.

Isolation of epothilone E and F

To isolate epothilone E and F, the shaking flask batches from the biotransformation (see above) are combined and are shaken for 1 hour with 20 mL of XAD-16. The XAD is recovered by sieving and is eluted with 200 mL of methanol. The eluate is evaporated in vacuum to 1.7 g crude extract. This is then partitioned between 30 mL of ethyl acetate and 100 mL of water. Upon evaporation in vacuum, 330 mg of an oily residue is obtained from the ethyl acetate phase. This is chromatographed in five runs through a 250 x 20 mm RP-18 column (solvent: methanol/water 58:42, detection 254 nm).

Yield:	Epothilone E	50 mg
	F	10 mg

Biological effect of epothilone E:

Using cell cultures, the concentration which reduces growth by 50% (IC_{50}) was determined, and the results were compared with the values for epothilone A.

<u>cell line</u>	<u>IC_{50} (ng/mL)</u>	
	<u>epothilone E</u>	<u>epothilone A</u>
HeLa. KB-3.1 (human)	5	1
mouse fibroblasts, L929	20	4

Epothilone E

$C_{26}H_{39}HO_7S$ [509]

ESI-MS: (positive ions): 510.3 for $[M+H]^+$

TLC: $R_f = 0.58$

TLC: aluminum foil 60 F 254 Merck. Solvent: dichloromethane/methanol = 9:1

Detection: UV absorption at 254 nm. Spraying with vanillin-sulfuric acid reagent; blue-gray coloration upon heating to 120°C

HPLC: $R_t = 5.0$ min

Column: Nucleosil 100 C-18 7 μm , 250 x 4 mm

Solvent: methanol/water = 60:40

Flow rate: 1.2 mL/min

Detection: diode array

1H -NMR (300 MHz, $CDCl_3$): δ = 2.38 (2- H_a), 2.51 (2- H_b), 4.17 (3-H), 3.19 (6-H), 3.74 (7-H), 1.30 - 1.70 (8-H, 9- H_2 , 10- H_2 , 11- H_2), 2.89 (12-H), 3.00 (13-H), 1.88 (14- H_a), 2.07 (14- H_b), 5.40 (15-H), 6.57 (17-H), 7.08 (19-H), 4.85 (21- H_2), 1.05 (22- H_3), 1.32 (23- H_3), 1.17 (24- H_3), 0.97 (25- H_3), 2.04 (27- H_3)

Epothilone F

$C_{27}H_{41}NO_7S$ [523]

ESI-MS: (positive ions): 524.5 for $[M+H]^+$

TLC: $R_f = 0.58$

TLC: aluminum foil 60 F 254 Merck. Solvent: dichloromethane/methanol = 9:1

Detection: UV absorption at 254 nm. Spraying with vanillin-sulfuric acid reagent; blue-gray coloration upon heating to 120°C

HPLC: $R_t = 5.4$ min

Column: Nucleosil 100 C-18 7 μm , 250 x 4 mm

Solvent: methanol/water = 60:40

Flow rate: 1.2 mL/min

Detection: diode array

$^1\text{H-NMR}$ (300 MHz, CDCl_3): δ = 2.37 (2- H_a), 2.52 (2- H_b), 4.20 (3-H), 3.27 (6-H), 3.74 (7-H), 1.30 - 1.70 (8-H, 9- H_2 , 10- H_2 , 11- H_2), 2.78 (13-H), 1.91 (14-H), 2.06 (14- H_b), 5.42 (15-H), 6.58 (17-H), 7.10 (19-H), 4.89 (21- H_2), 1.05 (22- H_3), 1.26 (23- H_3), 1.14 (24- H_3), 0.98 (25- H_3), 1.35 (26- H_3), 2.06 (27- H_3).

Example 4:

Preparation of epothilone E and F by biotransformation with *Sorangium cellulosum* So ce90

1) Carrying out the biotransformation

A culture of *Sorangium cellulosum* So ce90, which was shaken for four days in the presence of 2% XAD 16 adsorber resin (Rohm und Haas, Frankfurt/M.) at 30°C and 160 rpm, was used for the biotransformation. The culture medium has the following composition in g/liter of distilled water: potato starch (Maizena), 8; glucose (Maizena) 8; defatted soy meal, 2; yeast extract (marcor), 2; ethylenediaminetetraacetic acid, iron(III) sodium salt, 0.008; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 1; HEPES 11.5. The pH value is adjusted to 7.4 with KOH before autoclaving. The XAD is separated from the culture by sieving through a stainless steel screen (mesh size 200 μm). The bacteria are sedimented by centrifuging for 10 minutes at 10,000 rpm and the pellet was resuspended in 1/5 of the culture supernatant. Now, epothilone A or epothilone B in methanolic solution is added to the concentrated bacterial suspension at a concentration of 0.5 g/L. The culture is cultured further as described above. For the analysis of biotransformation, at the desired times, a 1 mL sample is taken, to which 0.1 mL of XAD is added and then the sample is shaken for 30 minutes at 30°C. The XAD is eluted with methanol. The eluate is evaporated to dryness and taken up again in 0.2 mL of methanol. This sample was analyzed by HPLC.

Figure 4) Kinetics of the biotransformation of epothilone A to epothilone E

Figure 5) Kinetics of the biotransformation of epothilone B to epothilone F

2) Preparation of epothilone E by biotransformation of 1 g of epothilone A

The strain *Sorangium cellulosum* So ce90 is cultured for four days in 8.5 L of the above medium (but without the addition of XAD) in a 10 liter bioreactor at 30°C at a rate of rotation of 150 rpm and with aeration of 0.1 vvm.

Then the culture is concentrated to 3 L by cross flow filtration. A membrane with a pore size of 0.3 μm and an area of 0.6 m² was used for this purpose.

The concentrated culture is transferred into a 4 liter bioreactor and a methanolic solution of 1 g of epothilone A in 10 mL of methanol is added. Then the culture is cultured further over a period of 21.5 h. The temperature is 32°C, the stirrer rotation rate 455 rpm and the aeration is done at a rate of 6 L/min. At the time of harvesting, 100 mL of XAD is added and the mixture is incubated further for 1 hour. The XAD is separated from the cells with a screen and then eluted exhaustively with methanol. The concentrated eluate is analyzed by HPLC.

Balancing of the biotransformation:

epothilone A used:	1000 mg = 100%
epothilone A found after 21.5 h:	53.7 mg = 5.4%
epothilone E formed after 21.5 h:	661.4 mg = 66.1%
epothilone A completely degraded:	= 28.5%

Experiment 5:

The epothilones according to the invention are tested with cell cultures (Table 2) and for promoting polymerization (Table 3).

Table 2:

Epothilone test with cell cultures

epothilone	A	B	C	D	E	F
	493	507	477	491	509	523
IC-50 (ng/mL)						
mouse fibroblasts L 929	4	1	100	20	20	1.5
human tumor cell lines:						
HL-60 (leukemia)	0.2	0.2	10	3	1	0.3
K-562 (leukemia)	0.3	0.3	20	10	2	0.5
U-937 (lymphoma)	0.2	0.2	10	3	1	0.2
KB-3.1 (cervical cancer)	1	0.3	20	12	5	0.5
KB-V1 (cervical cancer multires)	0.3	0.3	15	3	5	0.6
A-498 (kidney cancer)	-	1.5	150	10	20	3
A-549 (lung cancer)	0.7	0.1	30	10	3	0.1

Table 3:

Polymerization test with epothilones

Parameter: time to the half-maximum polymerization of the control

measurement	w	x	y	z	mean, [s]	mean, [%]
control	200	170	180	210	190	100
epothilone A	95	60	70	70	74	39
epothilone B		23	25	30	26	14
epothilone C	125	76	95	80	94	49
epothilone D	125	73	120		106	56
epothilone E	80	60	50	45	59	31
epothilone F	80	40	30	50	50	26

Standard test with 0.9 mg of tubulin/mL and 1 μ M of sample concentration

The polymerization test is an in-vitro test with purified tubulin from pig brain. The evaluation is done photometrically. Polymerization-promoting substances, such as epothilones, shorten the time elapsed till the half-maximum polymerization, that is, the shorter the time, the more effective the compound. w, x, y and z are four independent experiments and the relative effectiveness is expressed in the last column in % of the control; again, the lowest values correspond to the best effectiveness. Thus, the list corresponds quite accurately to that found in the cell cultures.

Our reference: 8824

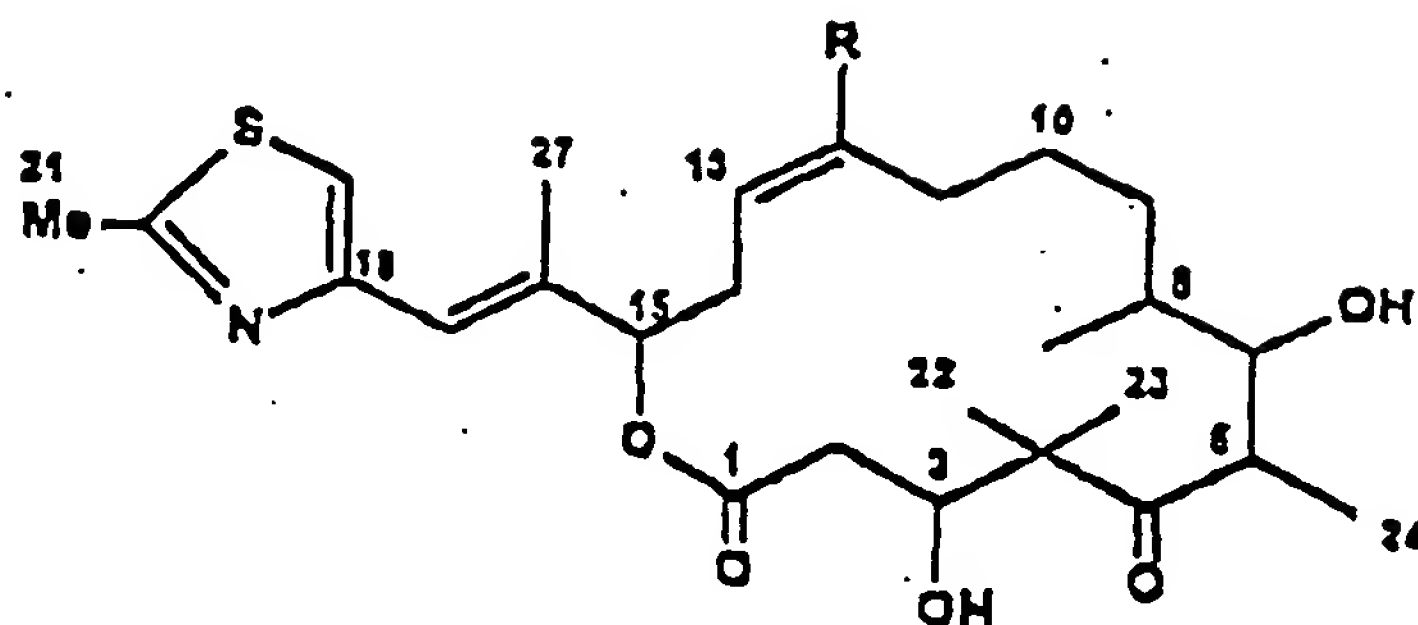
New International Patent Application PCT/EP

Gesellschaft für Biotechnologische Forschung mbH (GBF)

Patent Claims

1. Epothilones, obtainable by the fact that
 - (a) *Sorangium cellulosum* DSM 6773 is cultured in the presence of an adsorber resin in the known manner,
 - (b) the adsorber resin is separated from the culture and is washed with a water/methanol mixture,
 - (c) the washed adsorber resin is eluted with methanol and the eluate evaporated to a crude extract,
 - (d) the obtained concentrate is extracted with ethyl acetate, the extract is evaporated and partitioned between methanol and hexane,
 - (e) the methanolic phase is evaporated to a raffinate and the concentrate is fractionated on a Sephadex column,
 - (f) a fraction with metabolic products of the microorganism used is recovered,
 - (g) the recovered fraction is chromatographed on a C18-reverse-phase, with a methanol/water mixture and it is recovered in the time sequence
 - after a first fraction with epothilone A and
 - a second fraction with epothione [sic] B
 - a third fraction with a first additional epothilone and
 - a fourth fraction with a second additional epothilone and
 - (h1) the epothilone of the first additional fraction and/or
 - (h2) the epothilone of the second additional fraction is isolated.
2. Epothilone having the molecular formula $C_{26}H_{39}NO_5S$, characterized by the 1H and ^{13}C -NMR spectrum according to Table 1.

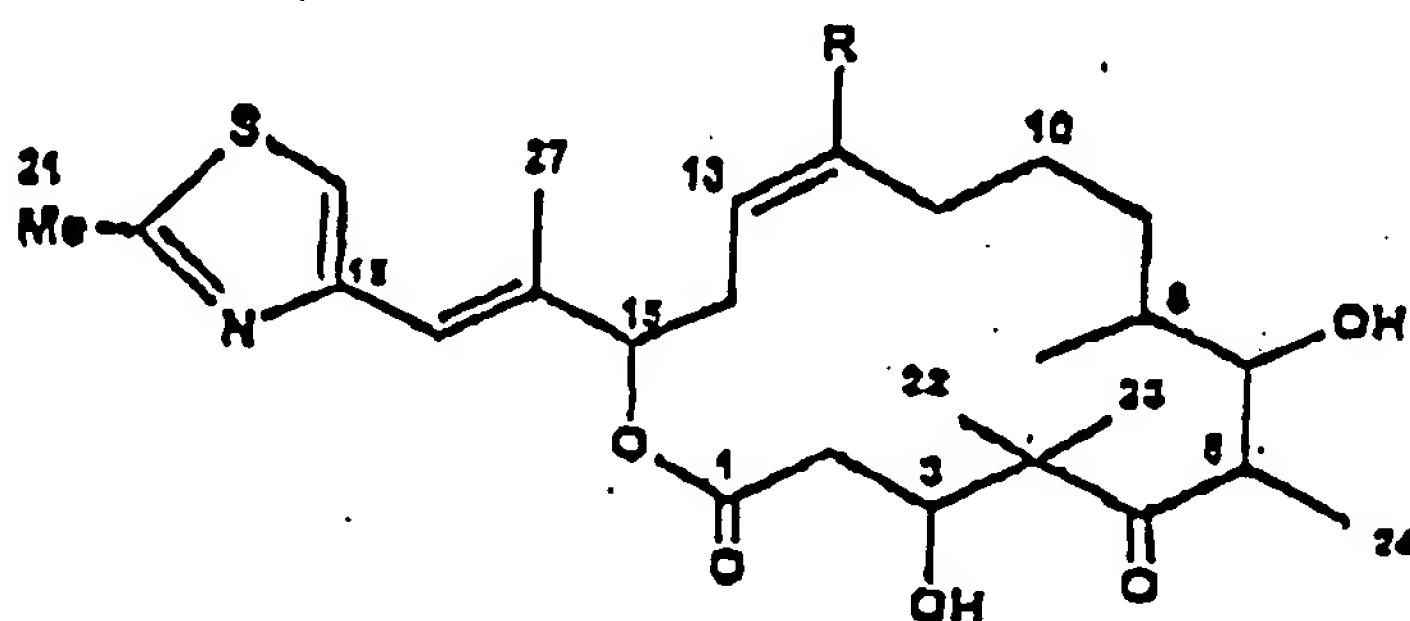
3. Epothilone C having the formula:



epothilone C R = H

4. Epothilone is the molecular formula $C_{27}H_{41}NO_5S$, characterized by the 1H and ^{13}C -NMR spectrum according to Table 1.

5. Epothilone D having the formula:



epothilone D R = CH_3

6. Biotransformant of epothilone A, obtainable by the fact that
 (a) *Sorangium cellulosum* DSM 6773 is cultured in the presence of an adsorber resin in the known manner, is separated from the adsorber resin and optionally the total amount or a part of the separated culture is treated with a methanolic solution of epothilone A,

- (b) the culture to which epothilone A was added is incubated and then an adsorber resin is added,
- (c) the adsorber resin is separated from the culture, eluted with methanol and the eluate is evaporated to a crude extract,
- (d) the crude extract is partitioned between ethyl acetate and water, the ethyl acetate phase is separated and evaporated to an oil,
- (e) the oil is chromatographed on a reverse-phase under the following conditions:
 - column material: Nucleosil 100 C-18 7 μ m
 - column dimension: 250 x 16 mm
 - solvent: methanol/water = 60:40
 - flow rate: 10 mL/min

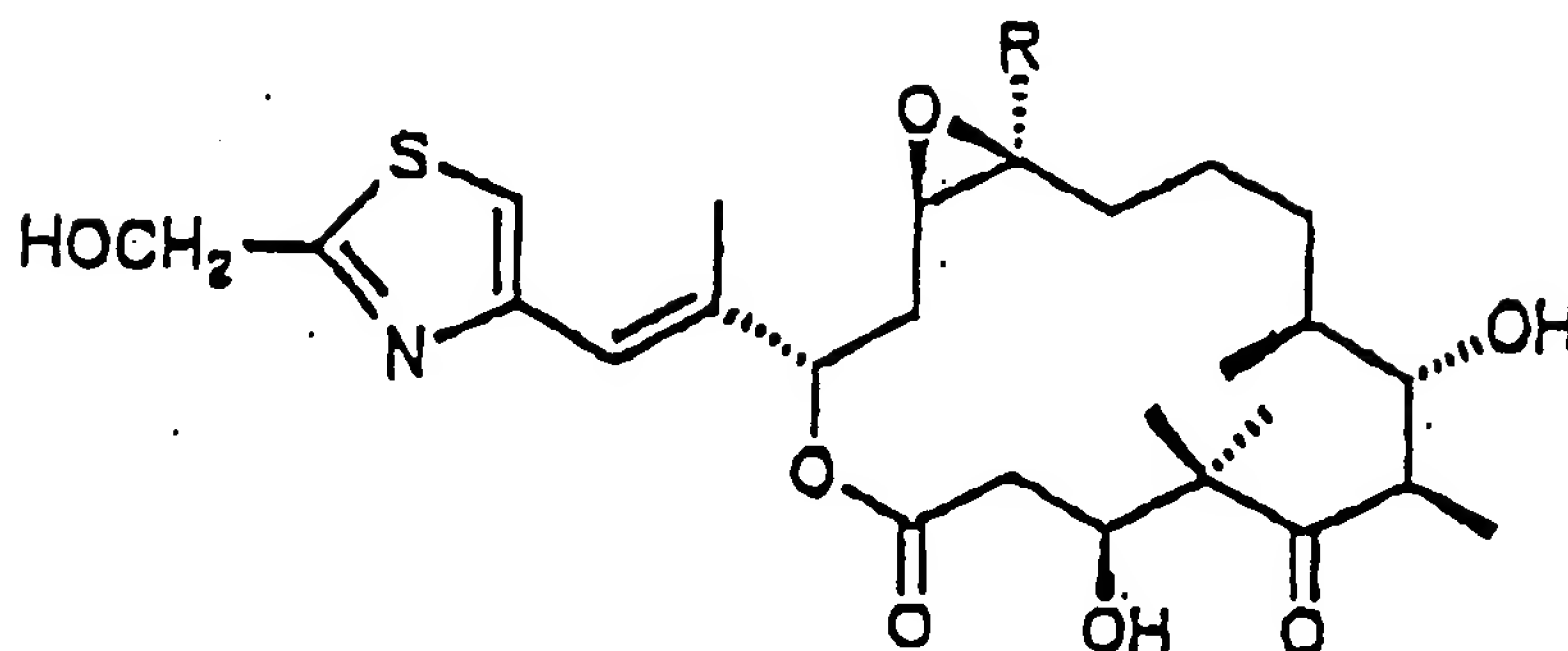
and fractions which contain a biotransformant, which can be detected by UV absorption at 254 nm, having an R_f value of 20 minutes, are separated and the biotransformant isolated.

7. Biotransformant of epothilone A according to Claim 6, obtainable by separating in step (a) a culture which is three or four or more days old.

8. Biotransformant of epothilone A according to Claim 6 or 7, obtained by incubating the mixture in step (b) for one or two or more days.

9. Compound having molecular formula $C_{26}H_{39}NO_7S$, characterized by the following 1H -NMR spectrum (300 MHz, $CDCl_3$): δ = 2.38 (2- H_a), 2.51 (2- H_b), 4.17 (3-H), 3.19 (6-H), 3.74 (7-H), 1.30-1.70 (8-H, 9- H_2 , 10- H_2 , 11- H_2), 2.89 (12-H), 3.00 (13-H), 1.88 (14- H_a), 2.07 (14- H_b), 5.40 (15-H), 6.57 (17-H), 7.08 (19-H), 4.85 (21- H_2), 1.05 (22- H_3), 1.32 (23- H_3), 1.17 (24- H_3), 0.97 (25- H_3), 2.04 (27- H_3).

10. Compound (epothilone E) having the formula:



epothilone E R = H

11. Biotransformant of epothilone B, obtainable by the fact that
- Sorangium cellulosum* DSM 6773 is cultured in the presence of an adsorber resin in the known manner, separated from the adsorber resin and optionally a methanolic solution of epothilone B is added to the total amount or to a part of the separated culture,
 - the culture to which the epothilone B was added is incubated and then adsorber resin is added,
 - the adsorber resin is separated from the culture, eluted with methanol and the eluate is evaporated to a crude extract,
 - the crude extract is partitioned between ethyl acetate and water, the ethyl acetate phase is separated and evaporated to an oil,
 - the oil is chromatographed on a reverse-phase under the following conditions:
column material: Nucleosil 100 C-18 7 μ m
column dimension: 250 x 16 mm
solvent: methanol/water = 60:40
flow rate: 10 mL/min

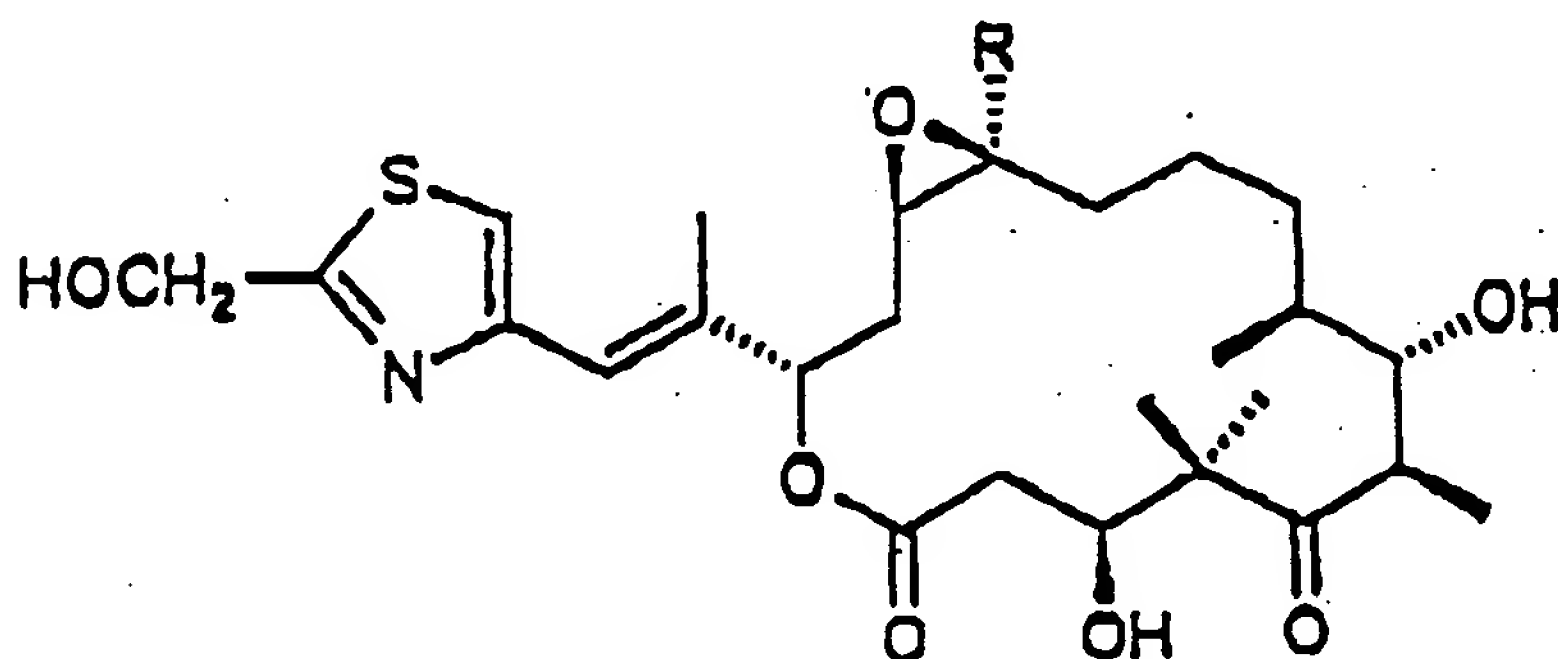
and fractions with a biotransformant content, which can be detected by UV absorption at 254 nm, with an R_f value of 24.5 minute, are separated and the biotransformant isolated.

12. Biotransformant according to Claim 11, obtainable by separating a culture in step (a), which is three or four or more days old.

13. Biotransformant according to Claim 11 or 12, obtainable by incubating the mixture at step (b) for one or two or more days.

14. Compound having the molecular formula $C_{27}H_{41}NO_7S$, characterized by the following 1H -NMR spectrum (300 MHz, $CDCl_3$): $\delta = 2.37$ (2- H_a), 2.52 (2- H_b), 4.20 (3-H), 3.27 (6-H), 3.74 (7-H), 1.30-1.70 (8-H, 9- H_2 , 10- H_2 , 11- H_2), 2.78 (13-H), 1.91 (14-H), 2.06 (14- H_b), 5.42 (15-H), 6.58 (17-H), 7.10 (19-H), 4.89 (21- H_2), 1.05 (22- H_3), 1.26 (23- H_3), 1.14 (24- H_3), 0.98 (25- H_3), 1.35 (26- H_3), 2.06 (27- H_3).

15. Compound (epothilone F) having the formula:



epothilone F $R = CH_3$

16. Agent for plant protection in agriculture and forestry and/or gardening, consisting of one or several compounds according to one of the previous claims, or of one or several of these compounds in addition to one or several of the usual carrier(s) and/or diluent(s).

17. Therapeutic agent, especially for use as a cytostatic agent, consisting of one or several of the compounds according to one or several of the previous Claims, or of one or several of the previous compounds according to one or several of the previous Claims in addition to one or several of the usual carrier(s) and/or diluent(s).

Figure 1. HPLC analysis of an XAD eluate at the end of a fermentation
 Epothilon = Epothilone

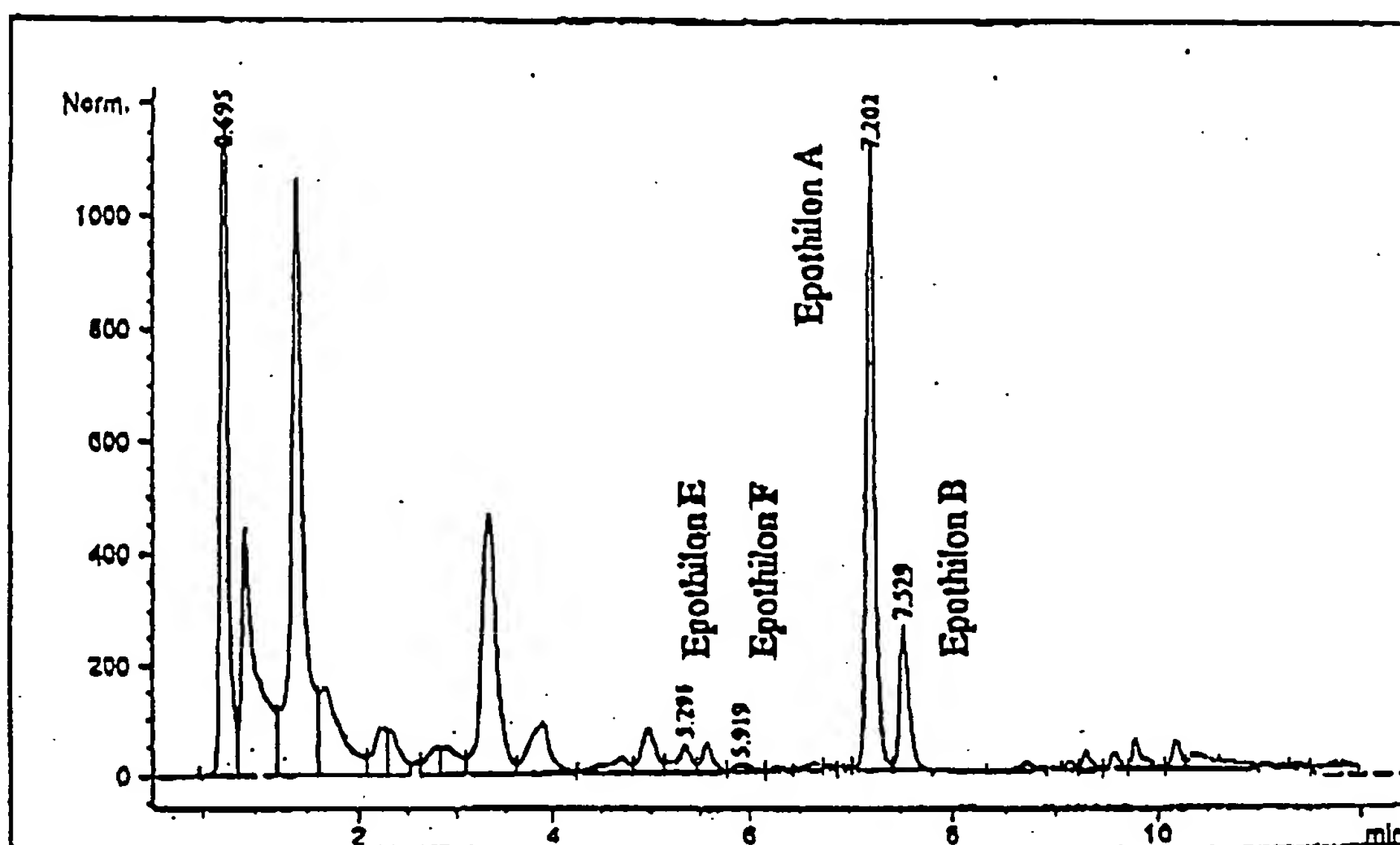


Figure 2. Enrichment of epothilone E and F in a fermentation broth after feeding a mixture of epothilone A and B, analyzed after 48 hour of incubation
 Epothilon = Epothilone

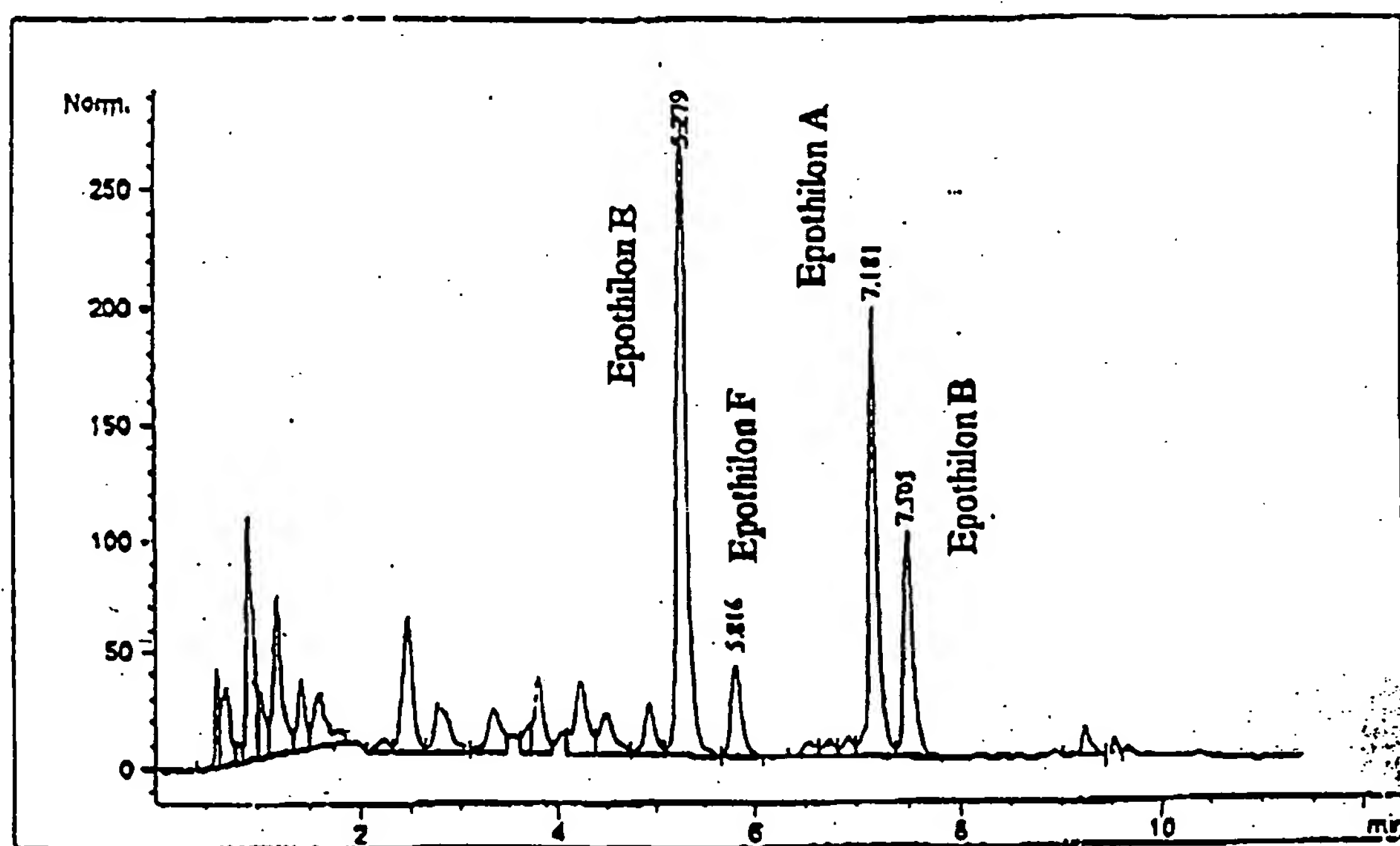


Figure 3. Kinetics of biotransformation of epothilone A to epothilone E by *Sorangium cellulosum* So ce90

Key:

Epothilon = Epothilone

on top: biotransformation of epothilone A

ordinate: epothilone [mg/L]

abscissa: incubation time [hours]

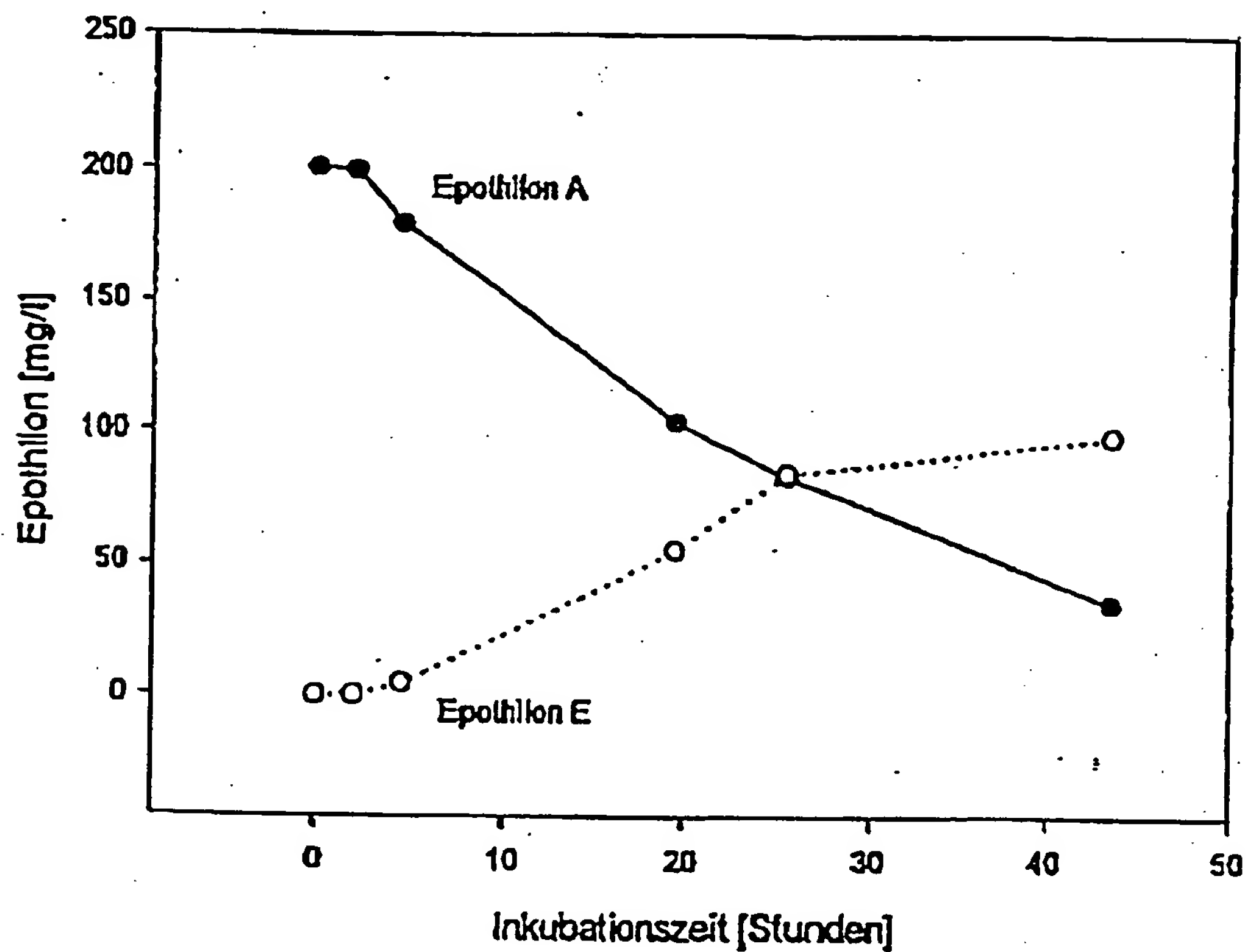


Figure 4

Key:

on top: biotransformation of epothilone A to epothilone E

inside the Figure: total epothilone
 epothilone A
 epothilone E

ordinate: epothilones [%]

abscissa: incubation time [h]

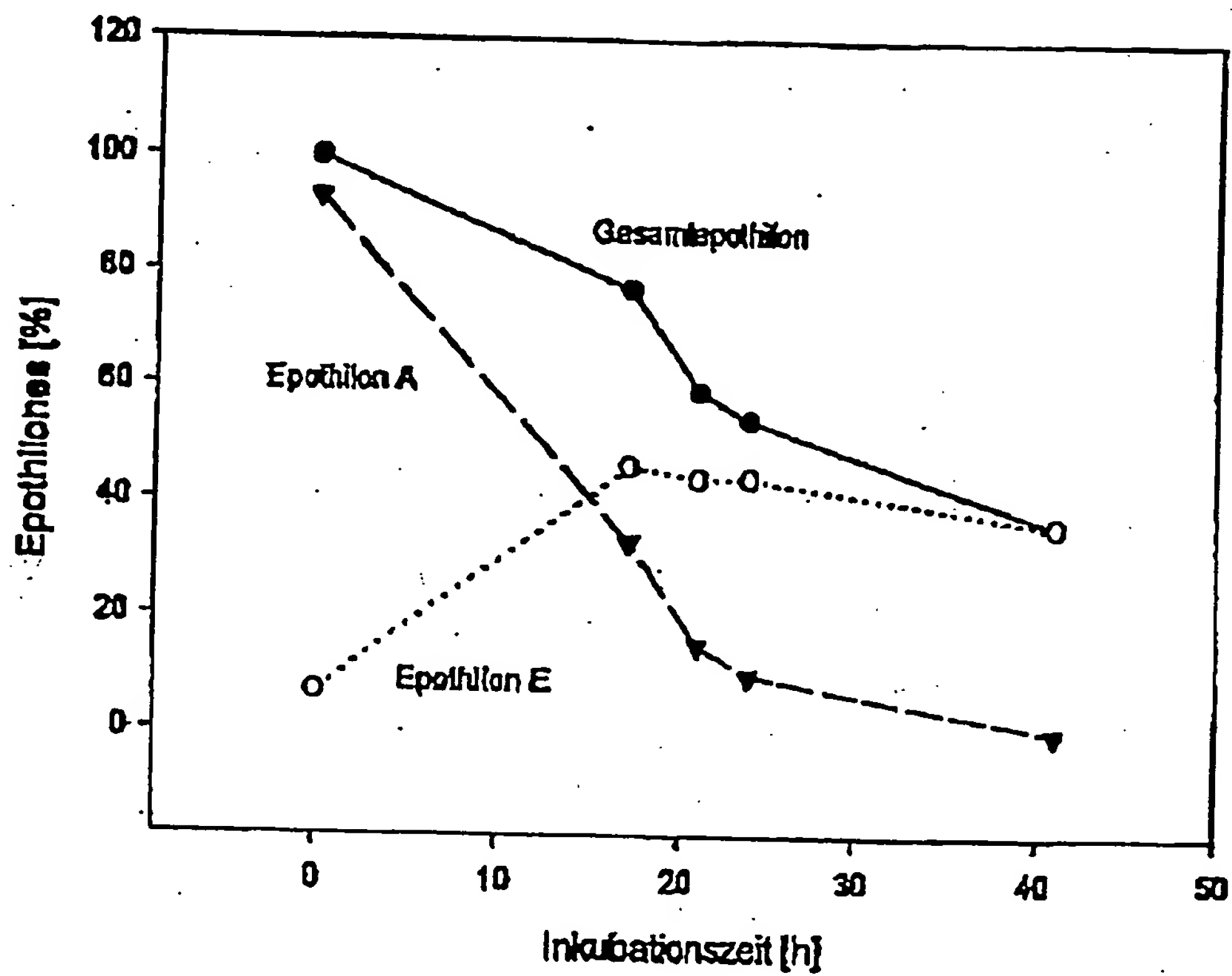


Figure 5

Key:

on top: biotransformation of epothilone B to epothilone E [corrected to F]

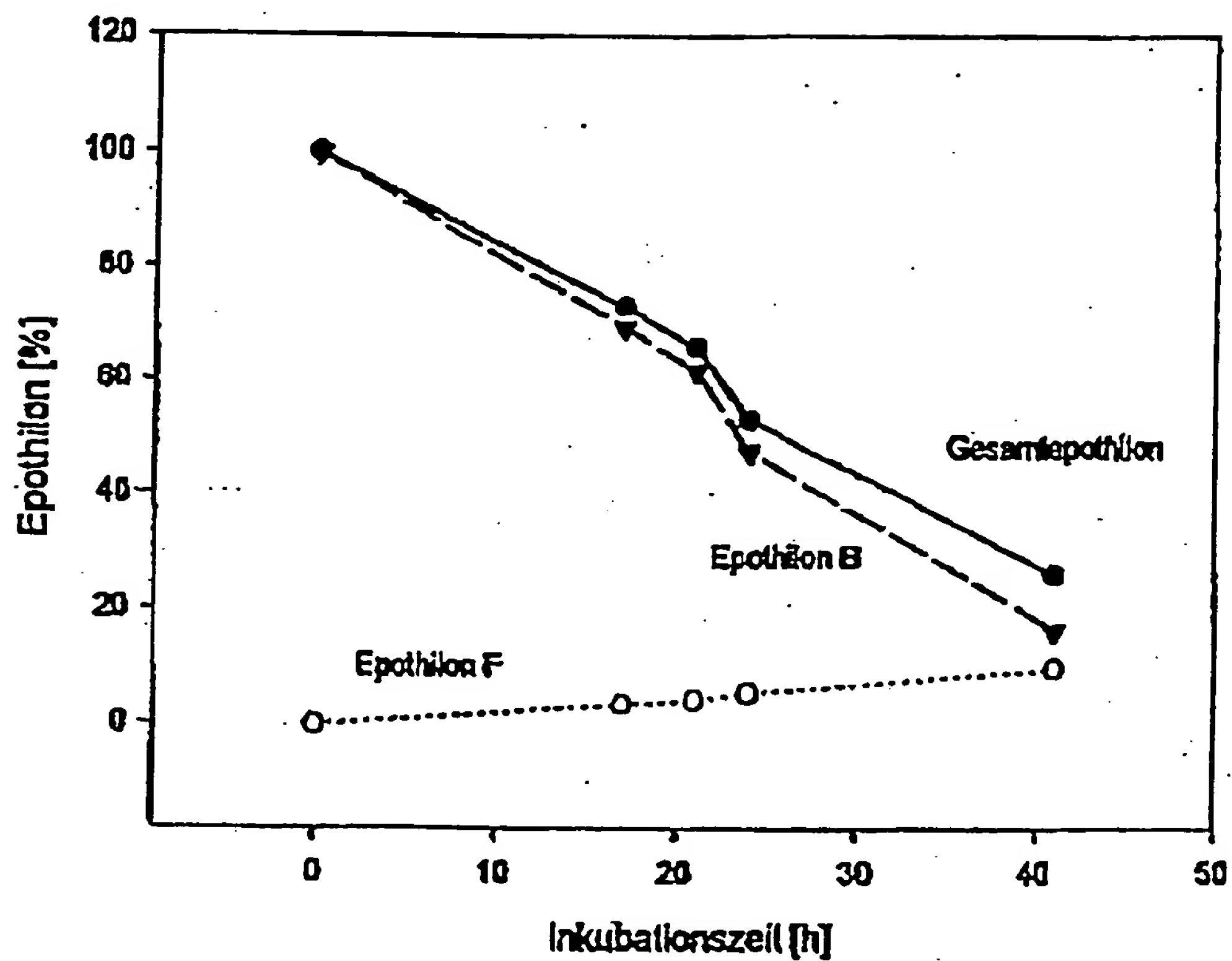
inside the Figure: total epothilone

epothilone B

epothilone F

ordinate: epothilones [%]

abscissa: incubation time [h]



November 17, 1997/he

Abstract

Our reference: 8824-GBF

New International Patent Application PCT/EP

based on DE (1) 96 47 580.5 and DE (1) 97 07 506.1

Gesellschaft für Biotechnologische Forschung mbH (GBF)

Epothilones C, D, E and F, preparation and agents

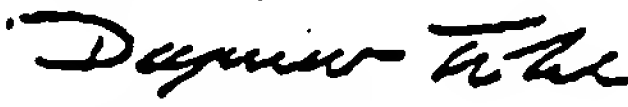
The present invention concerns epothilones C, D, E and F, their preparation as well as their application for the preparation of therapeutic agents and agents for plant protection

**BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE**

INTERNATIONAL FORM

**Gesellschaft für
Biotechnologische
Forschung mbH
Mascheroder Weg 1
3300 Braunschweig**

VIABILITY STATEMENT
Issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. DEPOSITOR		II. IDENTIFICATION OF THE MICROORGANISM	
Name: Gesellschaft für Biotechnologische Forschung mbH Address: Mascheroder Weg 1 3300 Braunschweig		Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM 6773 Date of the deposit or of the transfer ¹ 1991-10-28	
III. VIABILITY STATEMENT			
The viability of the microorganism identified under II above was tested on 1991-10-28 . ² On that date, the said microorganism was (<input checked="" type="checkbox"/>) ³ viable () ³ no longer viable			
IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED⁴			
IV. INTERNATIONAL DEPOSITARY AUTHORITY			
Name: DSMZ DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Mascheroder Weg 1 B D-3300 Braunschweig		Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):  Date: 1991-11-05	

¹ Indicate the date of original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).

² In the cases referred to in Rule 10.2(a) (ii) and (iii), refer to the most recent viability test.

³ Mark with a cross the applicable box.

⁴ Fill in if the information has been requested and if the results of the test were negative.

**BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE**

INTERNATIONAL FORM

Gesellschaft für
Biotechnologische
Forschung mbH
Mascheroder Weg 1
3300 Braunschweig

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
Issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
Identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR So ce 90	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM 6773
II. SCIENTIFIC DESCRIPTION AND/OR TAXONOMIC DESIGNATION	
The microorganism identified under I. above was accompanied by: <input checked="" type="checkbox"/>) a scientific description <input type="checkbox"/>) a proposed taxonomic designation (Mark with a cross where applicable)	
III. RECEIPT AND ACCEPTANCE	
This International Depositary Authority accepts this microorganism identified under I. above, which was received by it on 1991-10-28 (Date of original deposit) ¹	
IV. RECEIPT OF REQUEST FOR CONVERSION	
The microorganism identified under I. above was received by this International Depositary Authority on (date of original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).	
V. INTERNATIONAL DEPOSITARY AUTHORITY	
Name: DSM-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Mascheroder Weg 1 B 3300 Braunschweig	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): <i>Dieter Tügel</i> Date: 1991-11-05

¹Where Rule 7.1 applies, such date is the date on which the status of international depositary authority was acquired.

Epothilone E and F

Production strain:

The production strain *Sorangium cellulosum* So ce90 was isolated in July 1985 at GBF from a soil sample from the banks of the Zambesi and deposited on 10/28/91 at the Deutsche Sammlung für Mikroorganismen [German Collection for Microorganisms] under No. DSM 6773.

The characterization of the producing organisms and the culturing conditions are described in: Höfle, G.; N. Bedford, K. Gerth & H. Reichenbach: Epothilones, their preparation as well as agents containing them, DE 41 38042 A1, laid open on May 27, 1993.

Formation of epothilones E and E [sic] during fermentation:

Epothilone E and F, new biotransformation products of epothilones A and B

Production strain:

The production strain, *Sorangium cellulosum* So ce90, was isolated from a soil sample collected in July 1985 at the GBF at the banks of the Zambesi and was deposited on 10/28/91 in the Deutsche Sammlung für Mikroorganismen [German Collection for Microorganisms] under No. DSM 6773.

The characterization of the producing organism as well as the culturing conditions are described in:

Höfle, G.; N. Bedford, K. Gerth & H. Reichenbach: Epothilones, their methods of preparation as well as agents containing them. DE 41 38 042 A1, laid open on May 27, 1993.

Formation of epothilones E and E [sic] during fermentation:

A typical fermentation runs as follows: a 100 L bioreactor is filled with 60 L medium (0.8% starch; 0.2% glucose; 0.2% soy meal; 0.2% yeast extract; 0.1% $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$; 0.1% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$; 8 mg/L of Fe-EDTA; pH 7.4). In addition, 2% adsorber resin (XAD-16, Rohm & Haas) was added. The medium is sterilized by autoclaving (2 hours, 120°C). Inoculation is done with 10% of a preculture raised in a shaking flask in the same medium (in addition: 50 mM HEPES buffer pH 7.4) (160 rpm, 30°C). The fermentation is carried out at 32°C at a stirrer velocity of 500 rpm and aeration with 0.2 NL per m³ and hour. The

pH value is kept at 7.4 by the addition of KOH. The fermentation takes 7 to 10 days. The formed epothilones are bound to the adsorber resin continuously during the fermentation. After separation of the culture broth (for example, by sieving in a process filter) the resin is washed with 3 bed volumes of water and eluted with 4 bed volumes of methanol. The eluate is evaporated to dryness and is taken up in 700 mL of methanol.

HPLC analysis of the XAD eluate:

With respect to the initial volume of the reactor (70 L), the eluate is concentrated 100:1. The analysis is carried out with an HPLC unit 1090 made by Hewlett Packard. A Microbore column (125/2 Nucleosil 120-5 C₁₈) made by Machery-Nagel (Düren) is used for separating the components. The elution is done with a water/acetonitrile gradient from initially 75:25 to 50:50 after 5.5 minutes. This ratio is then maintained till the 7th minute and then increased to 100% acetonitrile up to the 10th minute.

The measurement is carried out at a wavelength of 250 nm and with a band width of 4 nm. The diode array spectra are measured in the wavelength region from 200 to 400 nm. In the XAD eluate, two new substances are noticed with R_f of 5.29 and R_f of 5.91; the adsorption spectra of these are identical to those of epothilones A and B, respectively (Figure 1; e corresponds to A, F corresponds to B). These substances are formed only in traces under the given fermentation conditions.

Biotransformation of epothilone A and B to epothilone E and F:

For the directed biotransformation, a 4-day old culture of *So ce90*, 500 mL, is used, kept with adsorber resin. Of this, 250 mL is introduced into a sterile 1 L Erlenmeyer flask leaving the XAD behind. After that, a methanolic solution of a mixture of a total of 50 mg of epothilone A + B is added and the flask is [incubated] for two days at 30°C and 200 rpm on a shaking chest [literal]. The formation of epothilone E and F is analyzed directly on 10 µL of the centrifuged culture supernatant (Figure 2). The conversion occurs only in the presence of the cells and is dependent on the cell density used and on the time. The kinetics of conversion for epothilone A is shown in Figure 3.

Isolation of epothilone E and F

To isolate epothilone E and F, the shaking flask contents from the biotransformation (see above) are combined and are shaken for 1 hour with 20 mL of XAD-16. The XAD is recovered by sieving and is eluted with 200 mL of methanol. The eluate is evaporated in vacuum to 1.7 g crude extract. This is then partitioned between 30 mL of ethyl acetate and

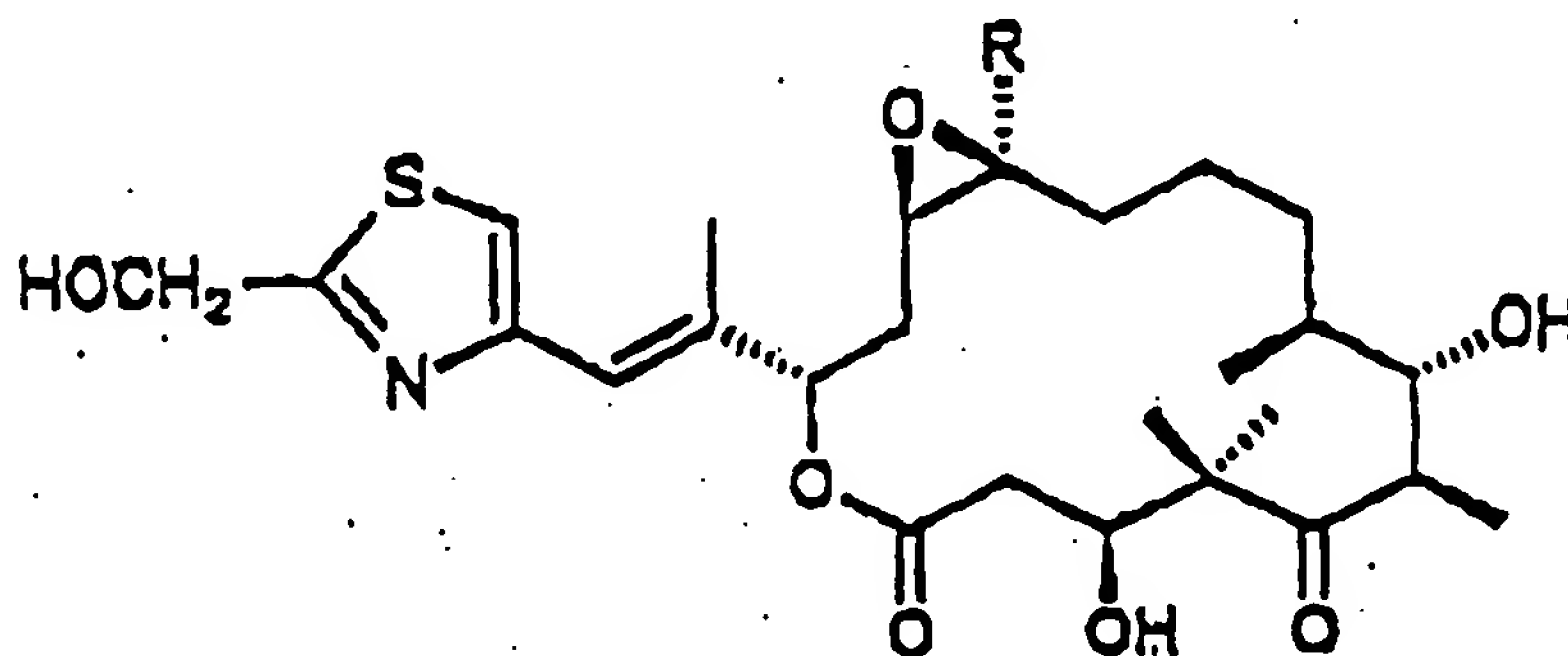
100 mL of water. Upon evaporation in vacuum, 330 mg of an oily residue is obtained from the ethyl acetate phase. This is chromatographed in five runs through a 250 x 20 mm RP-18 column (solvent: methanol/water 58:42, detection 254 nm).

Yield:	Epothilone E	50 mg
	F	10 mg

Biological effect of epothilone E:

Using cell cultures, the concentration which reduces growth by 50% (IC_{50}) was determined, and the results were compared with the values for epothilone A.

<u>cell line</u>	<u>IC_{50} (ng/mL)</u>	
	<u>epothilone E</u>	<u>epothilone A</u>
HeLa. KB-3.1 (human)	5	1
mouse fibroblasts, L929	20	4



Epothilone E $R = H$

Epothilone F $R = CH_3$

Epothilone E

$C_{26}H_{39}HO_7S$ [509]

ESI-MS: (positive ions): 510.3 for $[M+H]^+$

TLC: $R_f = 0.58$

TLC: aluminum foil 60 F 254 Merck. Solvent: dichloromethane/methanol = 9:1

Detection: UV absorption at 254 nm. Spraying with vanillin-sulfuric acid reagent; blue-gray coloration upon heating to 120°C

HPLC: $R_t = 5.0$ min

Column: Nucleosil 100 C-18 7 μm , 250 x 4 mm

Solvent: methanol/water = 60:40

Flow rate: 1.2 mL/min

Detection: diode array

1H -NMR (300 MHz, $CDCl_3$): δ = 2.38 (2- H_2), 2.51 (2- H_2), 4.17 (3- H), 3.19 (6- H), 3.74 (7- H), 1.30 - 1.70 (8- H , 9- H_2 , 10- H_2 , 11- H_2), 2.89 (12- H), 3.00 (13- H), 1.88 (14- H_2), 2.07 (14- H_2), 5.40 (15- H), 6.57 (17- H), 7.08 (19- H), 4.85 (21- H_2), 1.05 (22- H_2), 1.32 (23- H_2), 1.17 (24- H_2), 0.97 (25- H_2), 2.04 (27- H_2)

Epothilone F

$C_{27}H_{41}NO_7S$ [523]

ESI-MS: (positive ions): 524.5 for $[M+H]^+$

TLC: $R_f = 0.58$

TLC: aluminum foil 60 F 254 Merck. Solvent: dichloromethane/methanol = 9:1

Detection: UV absorption at 254 nm. Spraying with vanillin-sulfuric acid reagent; blue-gray coloration upon heating to 120°C

HPLC: $R_t = 5.4$ min

Column: Nucleosil 100 C-18 7 μm , 250 x 4 mm

Solvent: methanol/water = 60:40

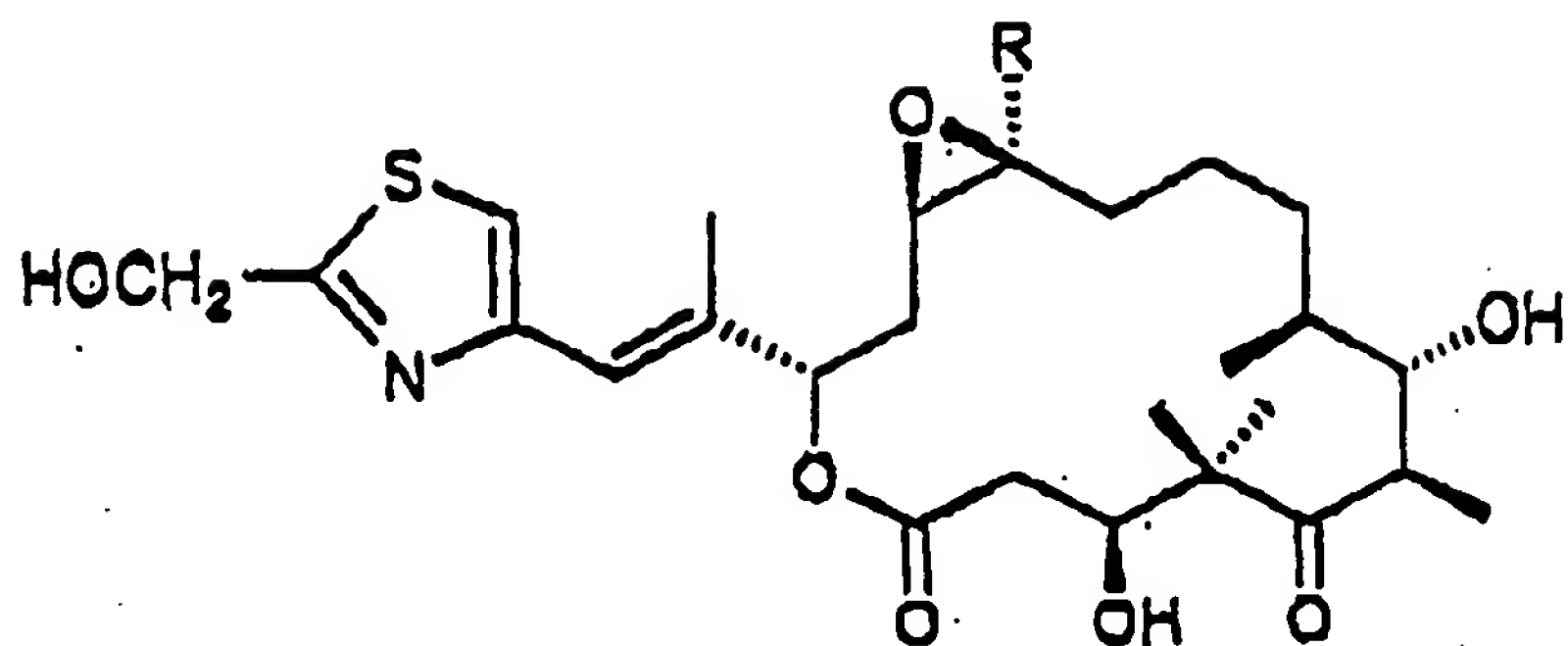
Flow rate: 1.2 mL/min

Detection: diode array

1H -NMR (300 MHz, $CDCl_3$): δ = 2.37 (2- H_2), 2.52 (2- H_2), 4.2 (3- H), 3.27 (6- H), 3.74 (7- H), 1.30 - 1.70 (8- H , 9- H_2 , 10- H_2 , 11- H_2), 2.78 (13- H), 1.91 (14- H_2), 2.06 (14- H_2), 5.42 (15- H), 6.58 (17- H), 7.10 (19- H), 4.89 (21- H_2), 1.05 (22- H_2), 1.26 (23- H_2), 1.14 (24- H_2), 0.98 (25- H_2), 1.35 (26- H_2), 2.06 (27- H_2)

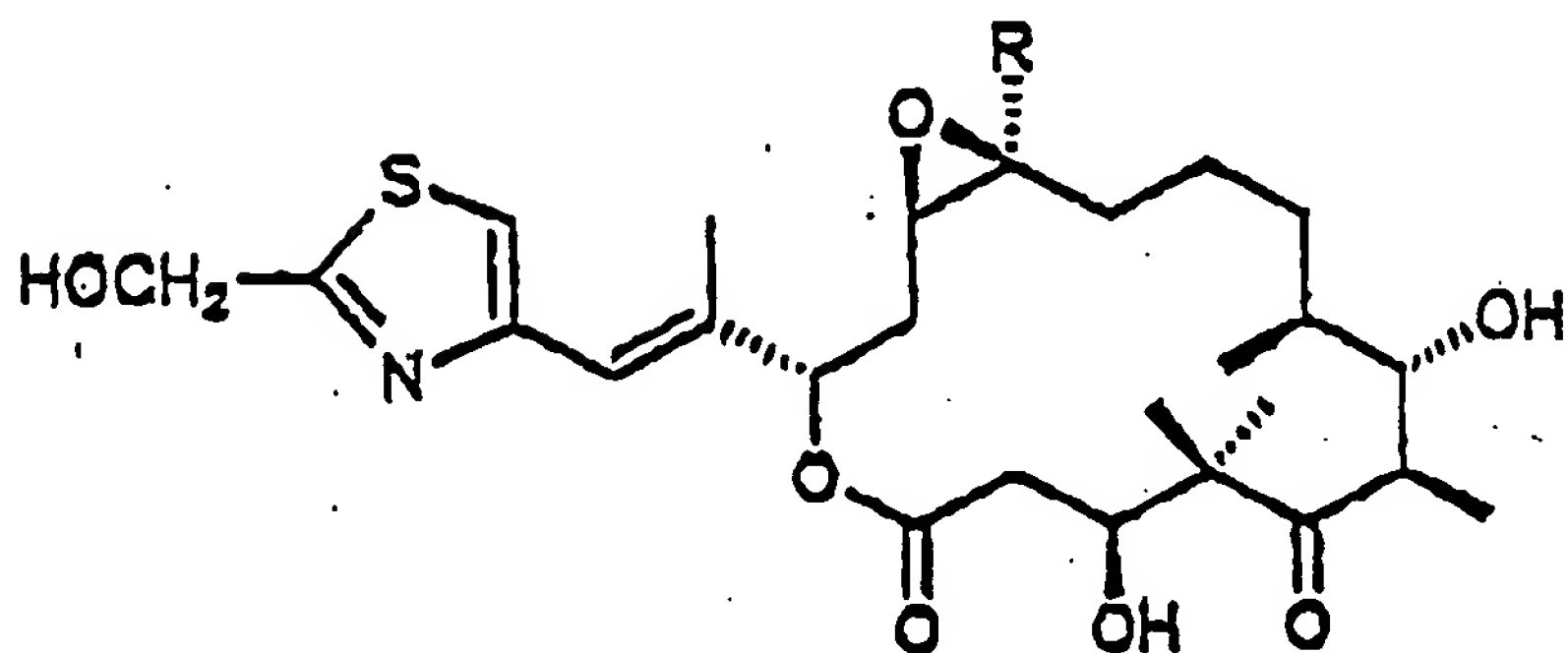
Patent Claims

1.



Epothilone E $R = H$

2.



Epothilone F $R = CH_3$

Figure 1. HPLC analysis of an XAD eluate at the end of a fermentation
 Epothilon = Epothilone

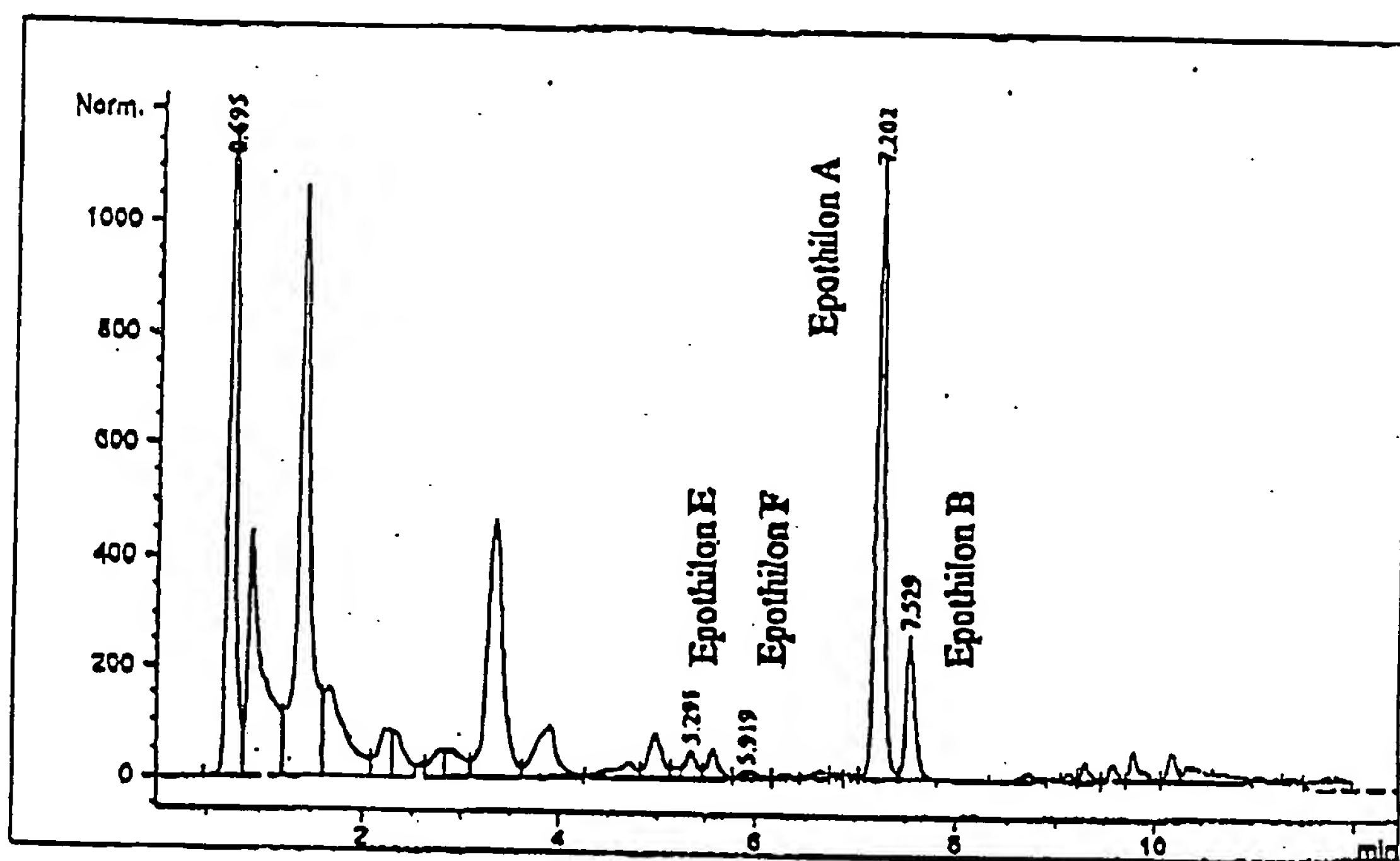


Figure 2. Enrichment of epothilone E and F in a fermentation broth after feeding a mixture of epothilone A and B, analyzed after 48 hour of incubation
 Epothilon = Epothilone

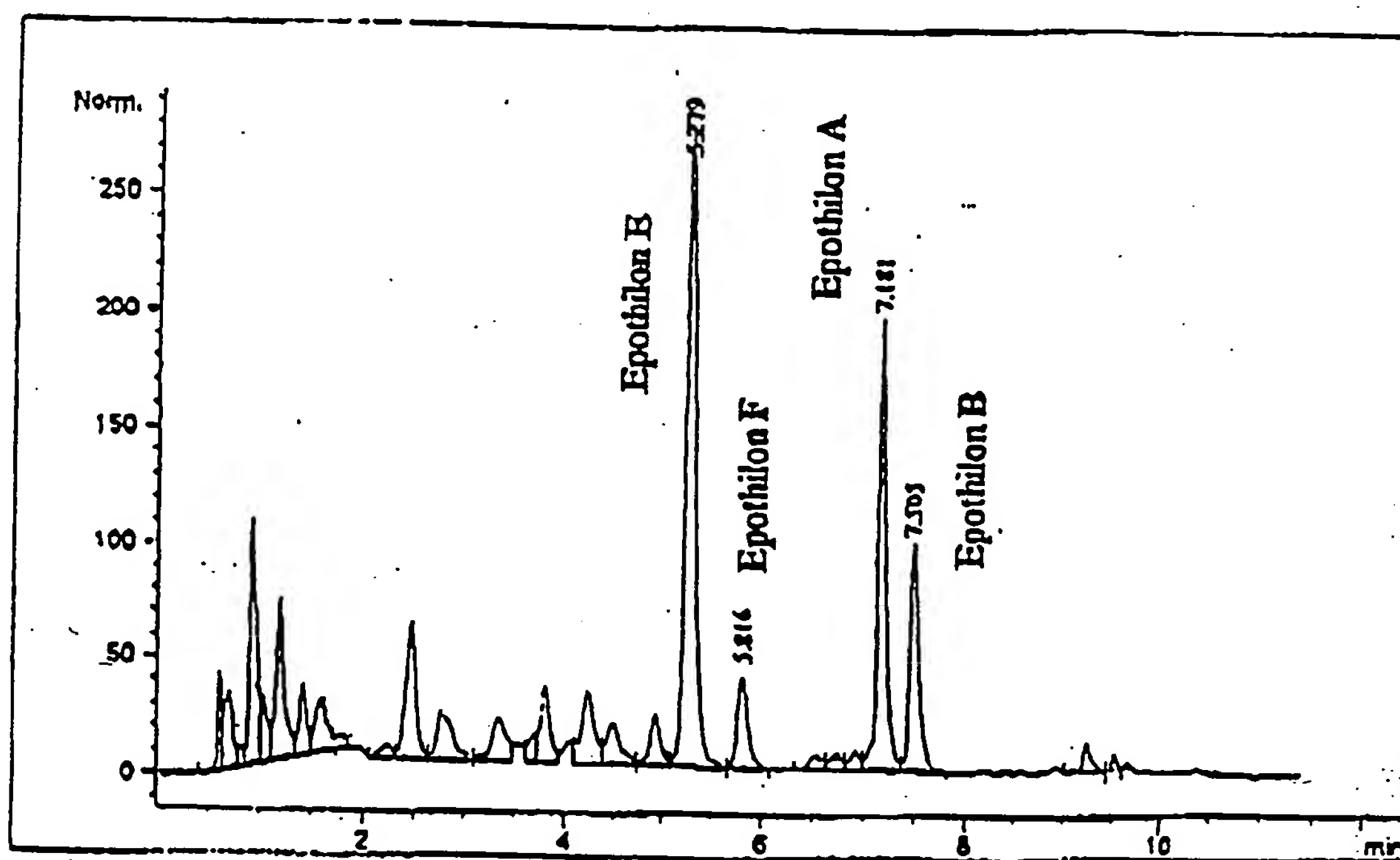


Figure 3. Kinetics of biotransformation of epothilone A to epothilone E by *Sorangium cellulosum* So ce90

Key:

Epothilon = Epothilone

on top: biotransformation of epothilone A

ordinate: epothilone [mg/L]

abscissa: incubation time [hours]

